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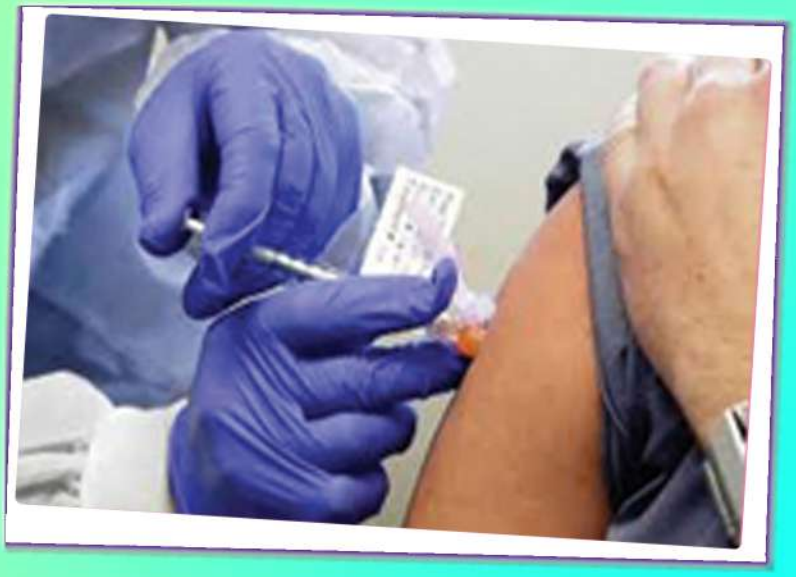

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Editorial

Nothing is Simple

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The emergence of the coronavirus disease-2019 (COVID-19) pandemic has given an unrequested, rare, and prominent moment to the discipline of Epidemiology. The rigors and tools of epidemiology are critical in such situations, as public health threats progress from misunderstood or poorly understood to managed to possible eradication. Those trained well can hold the dual levers of scientific knowledge and public office at the same moment and steer societies from fear and suspicion when outbreaks occur to a sense of calm accomplishment and resumption of "life as normal." In these moments epidemiology's true influence over the last 200 years is evident. However, no force can act alone.

It is often said in business that "culture beats strategy". The most beautifully crafted management plans can be derailed by attitudes, beliefs, and behaviors of groups. While epidemiology offers powerful tools for understanding the origin(s), population spread patterns and prediction by nature its focus is the population. And it depends upon the population's adherence to time-tested infectious disease precautions. Perhaps the modern-era and regretfully still challenging experience with the correct and consistent use of condoms in reducing Human Immunodeficiency Virus transmission provides a parallel example.

At this moment, the United States is experiencing a third "wave" of infections, if the first is counted. Hospitalizations and patient mortality rates are up and yet 50 million Americans and 90% of last year's Thanksgiving airline travelers planned travel. What is often apparent to epidemiologists or other scientists or clinicians is not always clear to the public. The nature and magnitude of risk posed by airline travel to family gatherings where multiple groups travel to be together is hard to know for sure but likely approaches levels unquantifiable by modern science. While policymakers and scientists pleaded with the public not to travel, many did so and a predictable increase in infections has followed. The Christmas holiday is approaching. Will we decide to do the same again?

Vaccines have arrived and optimism abounds in the United States and in other countries. Perhaps the beginning of the end is here. Until it is, individual responsibility to the group to remain well and prevent infections will continue to be critical. And the rationality and clarity of Epidemiology will compete with the subjective, shifting winds of culture.

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Review

Strategies and Challenges in the Development of Coronavirus Disease-2019 Vaccine

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ABSTRACT

The novel coronavirus infection (coronavirus disease-2019 (COVID-19)) emerged from Wuhan in the Hubei Province of China in late 2019. Millions of people were infected with COVID-19 pandemic due to the long incubation period of the virus inside the human body and the dearth of available treatments or vaccines. High transmission rates created havoc, which highlighted the urgent need for effective interventions to stop the spread and clinical impact of the virus on patients and populations. Previous research on severe acute respiratory syndrome coronavirus (SARS-CoV) provides information on vaccination strategies that could inform how governments approach the elimination of this novel coronavirus. Numerous efforts have been made to develop vaccines against Middle East respiratory syndrome (MERS) and SARS. The spike glycoprotein or S protein is the critical target for most of the drugs and vaccines against coronavirus. The virus uses the spike (S) protein for entering the host cell, by interacting with the receptor called angiotensin converting enzyme-2 (ACE2). Various vaccine platforms are available such as nucleic acid vaccine, protein-based vaccines, virus-vectored vaccines and live or attenuated vaccines, with each having their advantages and disadvantages. This review focuses on the overview of different vaccine candidates used, those currently in development, and the challenges encountered while developing effective vaccines.

Keywords
SARS-CoV-2; Vaccine development; Clinical trials.

INTRODUCTION

The outbreak of coronavirus disease-2019 (COVID-19) was first reported in Wuhan, Hubei Province of China in late 2019. The disease was caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which spread to almost all parts of the globe. The symptoms include fever, cough, chest tightness, and fatigue upon exertion. Most patients experience mild symptoms whereas some are asymptomatic (have no clear or confirmed symptoms).[1,2] Long incubation, high infection rates and mild to moderate symptoms make COVID-19 a troubling disease. The World Health Organization (WHO) declared this outbreak a pandemic on 30 January 2020. Physical distancing and other transmission mitigation strategies were implemented in most countries to prevent citizens from being infected. The SARS-CoV-1 outbreak in 2003 caused similar respiratory symptoms and amassed 774 deaths. Middle Eastern respiratory syndrome coronavirus (MERS-CoV) often involves similar symptomatology and infections in Saudi Arabia in 2012 were circulated between bats and camels before transmission to humans ("zoonotic" transmission).[3]

The coronavirus is an enveloped and positive sense single stranded ribonucleic acid (RNA) genome. It belongs to the *Betacoronavirus* genus containing 30 kb genome with 14 open reading frames (ORF). The ORFs includes four viral structural proteins: Membrane (M), Spike (S), Nucleocapsid (N) and Envelope (E) protein.[4-7] The S protein is functionally composed of two subunits, S1 (receptor binding) and S2 (cell membrane fusion).[8,9] During infections, host cell proteases process the S protein at the S1/S2 cleavage site. Proteolytically processed S protein cleaves into two subunits, the N-terminal at the S1 subunit and the C-terminal of the S2 subunit. The S1 subunit consists of N-terminal, signal peptide, and receptor binding domains. The S2 subunit consists of a C-terminal domain, conserved peptide sequences, proteolytic sites, a transmembrane domain and a cytoplasmic domain.[10-12] As the virus enters the host cell, another cleavage occurs for the fusion of

membranes and the cleavage is mediated by endolysosomal proteases.[8] Furin is highly expressed in lungs and the S protein contains the potential cleavage site for furin protease. Furin like cleavage appears to be the important for the activation of S protein which leads to the efficient entry of the virus into the host cell.[13]

Coronavirus (CoVs) can infect a range of host species such as animals, birds and humans. Different CoVs like SARS-CoV, MERS-CoV and SARS-CoV-2 have intricate host receptor recognition patterns, which indicate the structural diversity in the receptor binding domain of the S protein. The interaction between the S-protein and the angiotensin-converting enzyme 2 (ACE-2) receptor present on the host cell is the probable mechanism of the infection that is caused by SARS-CoV-2.[14]

According to WHO guidelines, the COVID-19 infected patients should receive supportive body system-focused therapies like fluid therapy, oxygen therapy and antibiotics. The major therapeutics drugs include lopinavir, ritonavir, remdesivir along with interferons, monoclonal antibodies and convalescent plasma for the treatment.[15] Remdesivir was originally developed for the treatment of Ebola virus infection. It prevents the viral infection by premature termination of RNA transcription.[16] Remdesivir is a nucleoside analogue drug which is in Phase III of clinical investigation against COVID-19 (NCT04292730, NCT04315948, NCT04257656). An open-label, randomized Phase II clinical trial found that triple antiviral therapy (lopinavir, ritonavir and interferon beta-1b) was safe in alleviating symptoms and shortening the duration of viral shedding. There is an unprecedented need to develop and distribute safe and effective vaccine to protect the entire global community from continued mortality from COVID-19 should be intact. The wide geographic diversity of the pandemic requires an effective vaccine approach, for this the collaboration between biotechnologist and pharmaceutical companies is needed which brings the convergence of a variety of vaccine development approaches. Over the past decade, the vaccine industry and scientific community responded urgently to several epidemics such as H1N1 influenza, Ebola, Nipah virus as well as against CoVs including MERS- and SARS-CoVs.[17-19] Developing vaccine against H1N1 influenza is relatively rapid as influenza vaccine technology is well-established and key regulators were already decided. Many efforts have been directed to develop vaccine against CoVs infection but the limiting factor is most often the degree of cross-reactivity.[20] Immune response by the body against SARS-CoV-2 vaccine plays a vital role for preventing the pathogen's entry into human cells. However, an unregulated immune response may lead to immunopathogenesis.

There is a challenge in developing safe and effective vaccines, but manufacturing, distributing and administering it to the population within a short time frame is an extraordinary challenge. Approximately, 321 COVID-19 vaccine candidates are available, out of these 66 vaccines are in activeclinical trials (Table 1) and 176 are in the pre-clinical (animal) phase of testing.[21]

An ideal vaccine should be safe even to immunocompromised people, inexpensive, free from toxicity, have high thermal stability and should confer long-term protection.[22] Various scientific communities are using multiple approaches to shorten the development phase which include efficiency gains *via* over-laying on one other of the traditional, sequential clinical "phases" of progressive testing prior to approval. This, while accomplishing research objectives more quickly than is usual, must be carefully monitored for rigor and reliability of conclusions reached at such previously-unexpected speeds. In addition to the more usual scientifically cautious approach to study and conclusion-drawing, these diverse types of vaccine candidates face a variety of challenges that are related to development, manufacturing, storage, and distribution, to mass vaccination.

VACCINE DESIGN STRATEGIES

The SARS-CoV-2 pandemic created a devastating situation across the globe.[14] Children and adults above 65-years of age are more vulnerable to COVID-19. Vaccination enable the natural viral infection defense system, which is the only way to control the COVID-19 outbreak. Vaccine design includes the selection of a target antigen key in the virus' infection process, a "vehicle" (viral, genetic/recombinant, chemical, etc.) for vaccine delivery into patients, and the intended or optimal vaccination administration route (oral, nasal, injection, etc.). The selection of a target antigen is based upon the structural and pathobiology information of SARS-CoV-2. The genome of SARS-CoV-2 is a single stranded positive sense RNA. The positive sense genome can act as messenger RNA that can be directly translated into viral protein by the host cell ribosome. The structural proteins present in the virus include Nucleocapsid (N) protein, spike (S) protein, membrane (M) protein and envelop (E) protein. N protein coats the positive stranded RNA genome which is enclosed in a lipid envelop into which S, M and E protein are inserted. The S protein located at the outer surface of virus binds with an ACE-2 receptor on the host cell surface, allowing receptor mediated endocytosis of the virus (Figure 1).[23] S protein plays an important role in the virus life cycle that enables it to enter human cells, so the spike protein is a rational priority prime target for most of the COVID-19 vaccines. Based on crystallography ACE2 binding patterns in SARS-CoV and SARS-CoV-2 are the same.[24] Previously, while developing vaccine against SARS-CoV, liver damage was observed when a full-length S protein was used as a vaccine antigen.[25] Thus, the fragment of S-protein, the receptor binding domain (RBD), was seen as a safer choice as a vaccine candidate for COVID-19. However, RBD-based vaccines face problems arising from low immunogenicity, identifying possible appropriate adjuvant(s), establishing an immunization schedule, and partial *versus* full fragment length approaches.[26] Vaccine candidates in development have several advantage and disadvantages. The most important features that should be considered to develop vaccine candidate are antigen-specific cellular immunity, long-term protection and the ability to induce a reliable and sufficient systemic immune system response.[27,28] Vaccine candidates for COVID-19 were classified into vector-based vaccines, recombinant protein-based vaccines, deoxyribonucleic acid (DNA) vaccines, messenger RNA (mRNA) vaccines, recombinant protein vaccines, inactivated vaccines, live attenuated vaccines and viral vector-based vaccines.

Deoxyribonucleic Acid Based Vaccine

Deoxyribonucleic acid vaccines are encoded with an antigen-pre-

Table 1. *Vaccine Candidates against COVID-19 in Development with Stage*

Candidate	Vaccine Characteristic	Sponsor/Lead Partner	Clinical Stage	Registration Number
DNA-Based Approach				
ZyCoV-D	DNA vaccine	ZydusCadila	Phase I/II	CTRI/2020/07/026352
INO-4800	DNA plasmid that encodes S protein delivered by electroporation	Inovio Pharmaceuticals	Phase I/II	NCT04336410
AG0301-COVID19	DNA vaccine that encodes S protein	Osaka University/ AnGes	Phase I/II	NCT04463472
GX-19	DNA vaccine that encodes S protein delivered by electroporation or needle free	Genexine Consortium	Phase I/II	NCT04445389
RNA-Based Approach				
mRNA-BNT162	LNP-encapsulated mRNA that encodes stabilised S antigen	Pfizer/BioNTech	Phase II/III	NCT04368728
mRNA	mRNA encoding the RBD	Walvax Biotechnology	Phase I	ChiCTR2000034112
mRNA-1273	LNP-encapsulated mRNA that encodes S protein	Moderna Therapeutics/NIAID	Phase III	NCT04470427
ARCT-021	LNP-encapsulated self-replicating mRNA that encodes the prefusion S protein	Arcturus Therapeutics	Phase I/II	NCT04480957
LNP-nCoVsaRNA	LNP-encapsulated self-amplifying RNA that encodes the S protein	Imperial College London	Phase I/II	ISRCTN17072692
CVnCoV	LNP-encapsulated mRNA that encodes the S protein	CureVac	Phase I	NCT04449276
Protein-Based Approach				
Covax-19	Recombinant SARS-COV-2 spike protein with Advax-SM adjuvant	Vaxine Pty/Medytox	Phase I	NCT04453852
SCB-2019	Recombinant SARS-CoV-2 trimeric S protein subunit vaccine	Clover Biopharmaceuticals	Phase I	NCT04405908
UQ-1-SARS-CoV2-Sclamp	Recombinant SARS-COV-2 spike protein 'molecular clamp' plus MF59 adjuvant	University of Queensland/CSL	Phase I	ACTRN12620000674932p
NVXCoV2373	Stable, prefusion protein, includes MatrixM™ adjuvant	Novavax	Phase II	NCT04368988
Adjuvantedrecombinant protein-based vaccine	Recombinant protein-based S protein vaccine together with ASO3	Sanofi / GSK	Phase I/II	NCT04537208
EpiVacCorona	Synthesized peptide antigens of SARS-CoV-2 proteins	FBRI SRC VB VECTOR	Phase I/II	NCT04527575
Coronavirus-like particle COVID-19 vaccine	Plant-derived virus-like particle with/without ASO3 or CPG1018 adjuvant	Medicago	Phase I	NCT04450004
Recombinant new coronavirus vaccine (CHO cell)	Recombinant SARS-CoV-2 RBD protein subunit vaccine	Anhui ZhifeiLongcom Biopharmaceutical/ IMCAS	Phase II	NCT04466085
Recombinant SARS-CoV-2 vaccine	Recombinant SARS-CoV-2 vaccine (Sf9 cell)	Sichuan University	Phase I	ChiCTR2000037518
Inactivated Virus Approach				
BBV 152	Whole-virion inactivated	Bharat Biotech, Indian Council of Medical Research, National Institute of Virology	Phase I/II	CTRI/2020/07/026300
Inactivated SARS-CoV-2 vaccine	Inactivated novel coronavirus (2019-CoV) vaccine	Beijing Institute of Biotechnology/ Sinopharm	Phase I/II	ChiCTR2000032459
Inactivated SARS-CoV-2 vaccine	Inactivated novel coronavirus Pneumonia vaccine (Vero cells)	Wuhan Institute of Biological Products/ Sinopharm	Phase III	ChiCTR2000034780
Inactivated SARS-CoV-2 vaccine	SARS-CoV-2 inactivated vaccine	Institute of Medical Biology, Chinese Academy of Medical Sciences	Phase I/II	NCT04470609
Adsorbed COVID-19 (inactivated) vaccine	SARS-CoV-2 inactivated vaccine	Sinovac Biotech	Phase III	NCT04456595
Viralvector-Based Approach				
V591	Measles virus vector	Merck Sharp &Dohme	Phase I	NCT04497298
Ad5-nCoV	Adenovirus type 5 vector that expresses S protein	CanSino Biological/ Beijing Institute of Biotechnology	Phase II	ChiCTR2000031781
Gam-COVID-Vac	Recombinant adenovirus vector based on the human adenovirus type 5, 26, containing S protein	Gamaleya Research Institute	Phase III	NCT04530396
GRAd-COV2	Gorilla adenovirus vector that expresses S protein	ReiTheraSrl	Phase I	NCT04528641
AZD1222	ChAdOx1 vector that expresses S protein	AstraZeneca/Oxford University	Phase III	NCT04516746
Ad26.COV2-S	Adenovirus type 26 vector that expresses S protein	J&J–Janssen	Phase I/II	NCT04436276
LV-SMENP-DC	DCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins	Shenzhen GIMI	Phase I/II	NCT04276896
Pathogen-specific aAPC	aAPCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins	Shenzhen GIMI	Phase I	NCT04299724

Figure 1. *SARS-CoV-2 Vaccine Strategy: S Protein of SARS-CoV-2 is the Target for Vaccine Development*

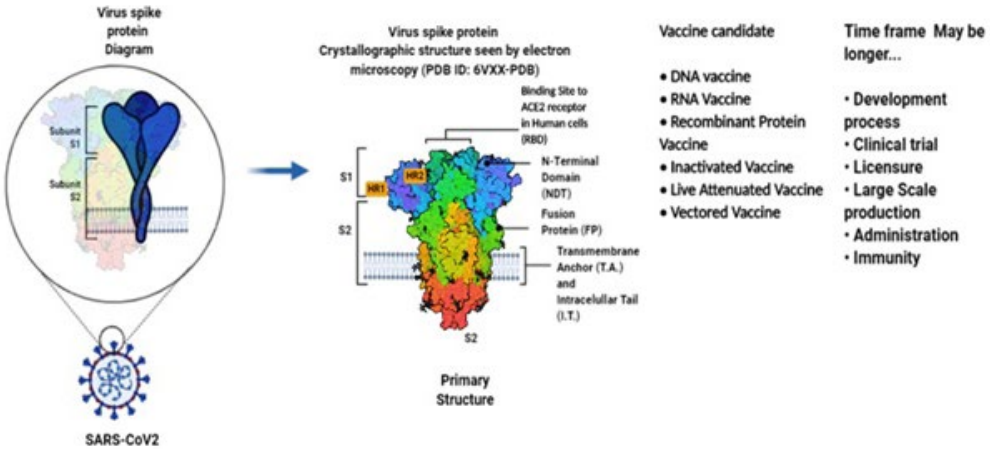
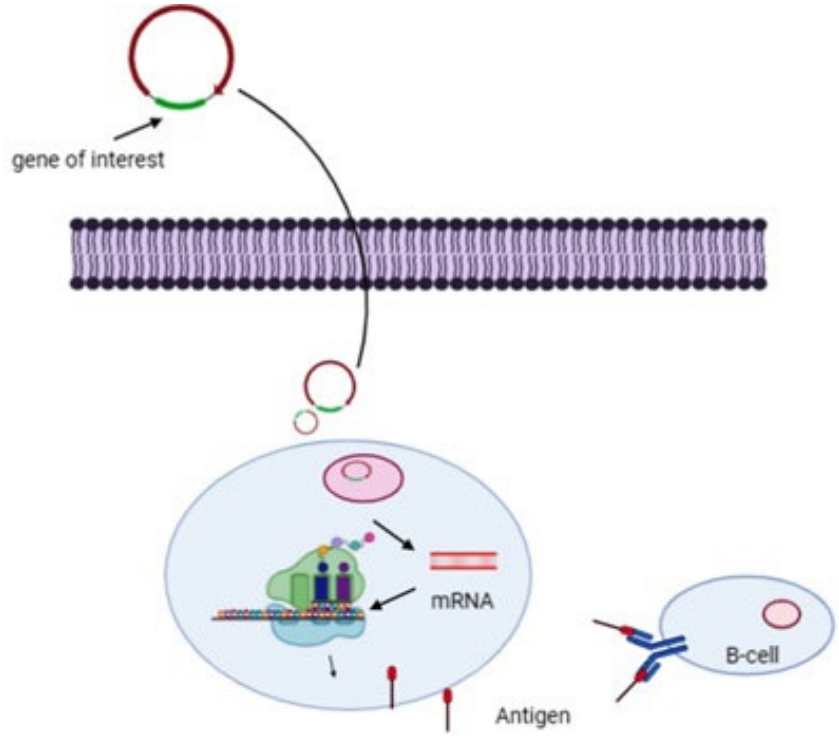


Figure 2. *Graphical Illustration of DNA-Based Vaccine Structure and Therapeutic Action*



senting cell which is intended to elicit humoral immune response that is like that of an atural infection.[29] The antigen-encoding DNA is enclosed with lipid nanoparticles can be delivered into the cytoplasm of the host cell and further it is processed into antigen peptide that elicits $CD8^+$ T-cell response.[30] DNA plasmids cross the plasma and nuclear membrane, enter the target cell, reach the nucleus and achieve transcription and thus propagates the desired and validated immune response (Figure 2). DNA vaccines have various advantages such as eliminating the use of live viruses, ease of production scaling, lower production costs when compared to protein vaccine production, more stability for storage and transportation and they may even be administered to immuno-compromised patients. Manufacturing DNA based vaccines is relatively straight forward and is more stable than viral protein and RNA vaccines. The only pro-inhibitory factor is the low immunogenicity that sometimes requires multiple booster doses.[31,32] Overcoming the immunogenic issues of DNA vaccines, immunostimulants and adjuvants maybe required. Combined use of granulocyte-macrophage colony-stimulating factor (GM-CSF) and a cytokine-based therapy directed against interleukin-4 (IL-4) have proven useful in enhancing immune response in some medical settings.[33] DNA vaccines show promising effects against several emerging viral dis-

eases such as Dengue, MERS, and Chikungunya.[34-36] Yet, no DNA vaccine has been licensed for use in human. DNA-based vaccines encoding S protein from SARS-CoV-2 appears to be in clinical Phase I/II testing on the pathway to approval.[37]

Ribonucleic Acid Based Vaccines

Messenger RNA (mRNA) sequences that code for disease specific antigens enclosed with lipid nanoparticles (LNPs) are the current, most widely pursued mechanism of action for approved COVID-19 vaccines.[38] Once the LNP is phagocytosed (eaten/incorporated) by the human cell, the RNA condensing lipid nanoparticle punctures the endosome and allows the mRNA molecule to release into the cytosolinside the human cell (Figure 3). Messenger RNA vaccines are synthesized by *in vitro* transcription and are non-infectious. This feature of mRNA vaccines does provide advantages that differentiate it from recombinant viral vector vaccines, live attenuated viral vaccines and inactivated viral vaccines that enable inexpensive and rapid production of vaccine doses.[39] The mRNA vaccine does not integrate permanently into the human host genome and does not produce any live infectious particles that elicit immune responses and this further reduces safety concerns.

Moderna's COVID-19 vaccine candidate mRNA-1273, encodes SARS-CoV-2 S protein encapsulated in lipid nanoparticles, and is in Phase III clinical trials.

Protein Vaccines

Protein vaccines contain partial or full-length SARS-CoV-2 S protein that induces a $CD4^+$ TH-cell and antibody response. Unlike nucleic acid-based vaccine, protein subunit vaccines could have improper epitope conformation, until they are produced by mammalian cells.[40] Protein vaccines are designed to provoke the immune response towards neutralizing epitopes, thus preventing the production of non-neutralizing antibodies that may promote antibody- dependent enhancement (ADE) of disease.[41] Protein subunits (when used alone) are poorly immunogenic and thus serve as poor activators of the $CD8^+$ T-cell response, which requires adjuvant and repeated administrations. Recombinant proteins are enclosed in a virus like particles (VLPs) devoid of viral genome. The VLPs generate high numbers of antigenic epitope copies, thereby preserving viral immunogenicity, enabling the ability to crosslink to B-cell receptors on the B-cell surface and facilitate uptake by the antigen presenting cells (APCs) (Figure 4).[42,43] In protein-based vaccines, subunits are taken up by the antigen presenting cells for B-cell recognition through B cell receptor (BCR) and for major histocompatibility complex (MHC) presentation. In viral-vector vaccines, (replicating and non-replicating) viral vectors or cellular vehicles, carrying a desired gene, are delivered and facilitate the expression of heterologous antigen within the cell. The viral vector retains the capacity to produce viral particles that infect the host cell and that lead to transgene expression and antigen presentation. Live virus vaccines retain the ability to replicate inside the host cell and mimic the natural infectionprocess. The inactivated virus vaccines deploy virus cells that were once alive and have been completely attenuated, yet still generate an infectious state. They do so by using certain chemicals that induce specific neutralizing antibodies in response to what the viral vaccine presents to the human body.

Figure 3. *Graphical Illustration of RNA Based Vaccine*

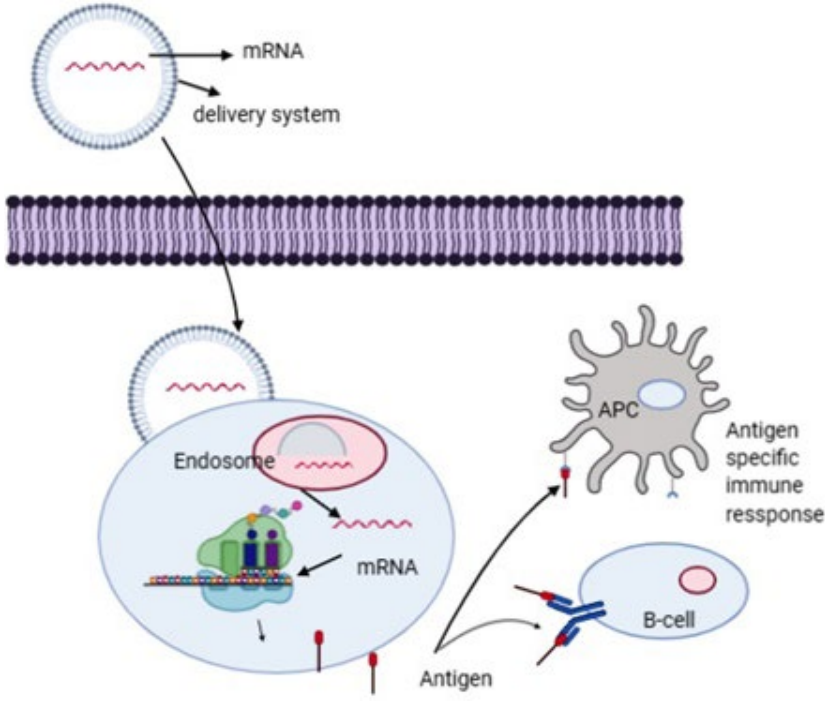
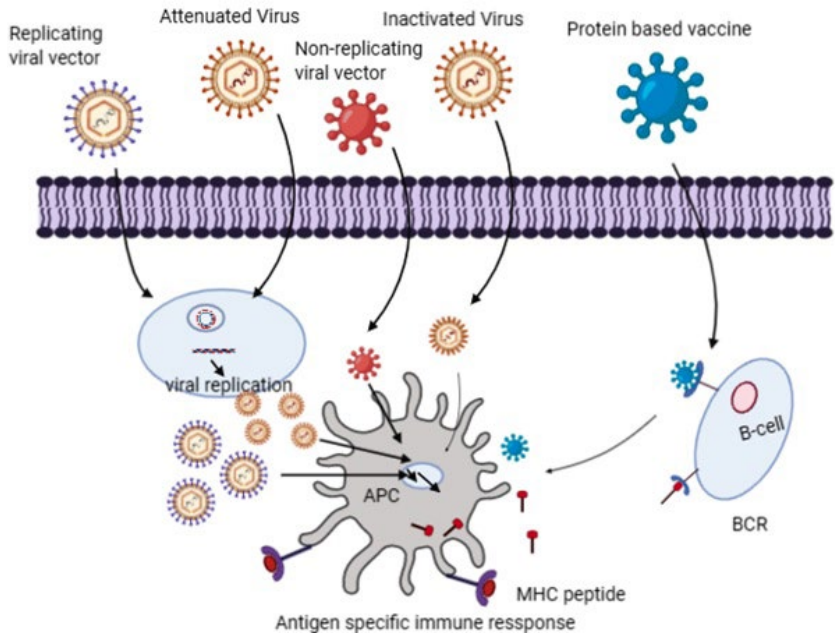


Figure 4. *Graphical Representation for Vaccine Design Strategies: Protein Based Vaccines-Subunits are Taken-up by the Antigen Presenting Cells for B-Cell Recognition through BCR and for MHC Presentation*



Viral vector vaccines (replicating and non-replicating) viral vector carrying a desired gene is delivered and allows the expression of heterologous antigen within the cell, the viral vector retains the capacity to produce viral particles that infect the host cell and leads to transgene expression and antigen presentation. Attenuated viruses retain the ability to replicate inside the host cell and mimic the natural infectionprocess. Inactivated virus generates an infectious state using certain chemicalsthatinduce specific neutralizing antibodies.

Attenuated and Inactivated Virus Vaccine

The attenuated vaccines are obtained by mutating a virus, which infects the human cell until a substantial number of cells adopt the mutation that generates the desired immune response (Figure 4). Their most important advantage is that they offer more than one antigenic component to host, thus inducing or mobilizing various immunological effectors against the virus.[44] The drawback associated with attenuated vaccines are potential safety concerns. They often have high reactogenicity compared to protein-based vaccines.[45] Several successful vaccines such as bacillus-calmette-guerin (BCG) and measles vaccines are based on attenuated strains of the viruses.[46] The coronavirus genome has various genes that are not required for replication and can be deleted. Deletion of various genes that code for non-structural proteins and structural E proteins may also be used as a strategy to design new vaccines.[45,47,48] Therefore, deletion of the virulence factor and the induced codon deoptimization (where nucleic acid sequence is modified that encodes the wild type of amino acid sequence, and slows the translation of viral protein) may be a suitable mechanism of attenuation. A codon deoptimization approach yields vaccine-ready virus that is highly attenuated *in vivo* and *in vitro* and can replicate if the correct viral protein(s) is/are selected forde-optimization.[49,50]

In inactivated vaccines, the whole pathogen is killed either by exposure to chemical (formaldehyde) or heat induced inactivation.[51] Inactivated viruses have been used traditionally for vaccine development and were found to be safe and effective for prevention of diseases caused by the hepatitis influenza, and polioviruses. They are generally less immunogenic and require multiple doses or an additional adjuvant (Figure 4).[52,53] Currently there are five vaccine candidates for SARS-CoV-2 (Table 1) apart from that there are nine inactivated vaccine that is in preclinical stage (WHO). Inactivated vaccine candidate (NCT04456595) developed by Sinovac Biotech Ltd in China, is currently in phase III clinical trials.[54]

Viral Vector Based Vaccines

Viral vector-based vaccines use either replicating or non-replicating viruses (Figure 4) to induce the human immune response. It represent the biotechnological evolution of inactivated and attenuated vaccines, which are viral backbones devoid of replication machinery and used as a vehicle to deliver *in vivo* and express antigens derived from target pathogens. The viral- based vaccine has ability to induce strong T-cell responses without the need forany adjuvants.[55,56] One of the drawbacks of viral vector vaccines is that it requires multiple immunizations to achieve the level of immunogenicity and thus protection against the virus but may also lead to ahost response against the structural viral protein, thus limiting the efficacy of immunization (called a neutralizing antibody).[55] This limitation is overcome by using aheterologous prime boost regimen that is introduced in several clinical trials.[57]

Heterologous prime-boost vaccine regimens means to deliver the same or similar antigens through different vaccine types, the first to prime and the second to boost the immune system.

Currently there are eight viral-based vaccine candidates

is in clinical trials (Table 1). Ad5-nCoV (NCT04341389) is a viral vector-based vaccine candidate that has been in Phase III of clinical trials. The replicating viral vaccine candidates are based ona vaccine strain derived from the human pathogen. It is important to consider this very specifically, if the potential recipient of the vaccine has pre-existing exposure and presumed subsequent immunity against the virus. Pre-existing antibodies can impair the ability of vaccine to elicit the immune response required for protection. Non-replication viral vector vaccine candidates are mostly based on adenovirus (Ad5) and express the S protein or receptor binding domain (RBD) of SARS-CoV-2.

Safety Measures for Coronavirus Disease-2019 Vaccines

COVID-19 cases do not seem to be declining and the ebb and flow of cases are mostly driven by human attitude towards physical distancing and protective measures. All eyes remain on pharmaceutical companies and research institute involved in vaccine development. Traditionally new vaccine development and approval requires 10-12-years, and there are enormous pressures and challenges to minimize that time frame. The safety of a vaccine is generally determined by the choice of adjuvant, nature of vaccine platform, mode and route of vaccine administration, status of pre-existing vaccine immunity and age of vaccines. In the particular case of SARS-CoV-2, the individual is either symptomatic or asymptomatic, and COVID-19 vaccines are developed with a goal of mass immunization or protection and not addressing symptomatology. Any loophole in the lack of safety considerations may support the mobilization of anti-vaccination movements or messaging, which would jeopardize the effect of achieving mass immunization.

As other respiratory viral infections, vaccine strategies for COVID-19 require additional safety vigilance. There is a possibility of antibody-dependent enhancement (ADE) of disease if an insufficient immune response is mounted in a situation of the unresolving, active, and serious disease. Over production of pro-inflammatory cytokines in lung immunopathology that cause additional damage when vaccines cannot stop SARS-CoV-2 pathology early in the process. In this regard, COVID-19 vaccine trials were initially conducted in healthy adults with age of 55-years or younger and later stage trial includes seniors.[58,59]

DISCUSSION

The COVID-19 pandemic emerged as wide spread SARS-CoV-2 infection in December 2019, according to most estimates. Several challenges exist in the development of vaccines against COVID-19 as the novel SARS-CoV-2 is undergoing several genomic changes, even as of the time of this writing. Developed vaccine candidates include approaches with inactivated viruses, live attenuated viruses, virus nucleic acid-based vaccine, and protein subunit vaccines. All are in different clinical phases. Moderna's vaccine; the inactivated virus vaccine being developed by Sinopharm, the Wuhan institute of Biological products; and Sinovac Biotech are on the market as of this writing. According to WHO Global Advisory Committee, AstraZeneca (adenovirus vector vaccine) is that, it is safe and effective for protecting people from the serious risk of COVID-19. The ideal vaccine candidate for COVID-19 should be safe, effective and have a good immunogenicity profile among all age groups and be safe for including pregnant women and immuno compromised individuals. For ease of success, the effective vaccine should generate humoral and cellular immunity with a single dose of vaccination. Generating effective vaccines has required initiating a large number of official projects by the WHO, various companies, universities and laboratories. Approval of the first COVID-19 vaccine in increased the enthusiasm and possibilities for developing second and third generation vaccine approaches and candidates for approval.[60,61] COVID-19 eradication programme is time taking after vaccination, as the various mutant strains are available now e.g. South Africa variant (501Y.V2), which show different symptoms in infected humans than previous strains.

CONCLUSION AND FUTURE GUIDEANCE

Various companies and institutes are in the position to offer several treatment strategies against the COVID-19 pandemic. Tireless and ongoing scientific efforts have led to the development of 242 COVID-19 vaccines candidates. We are in the early stage of SARS-CoV-2 identification and adequate vaccine preparation, manufacturing, and distribution to populations. Based on previous studies, it may be inferred that the COVID-19 vaccines approved thus far provide acceptable (if not higher than previously experienced) immunity and protection via a durable neutralizing antibody and a lasting T-cell response.

Various vaccine strategies are being pursued now with attendant advantages and disadvantages. According to the WHO, the vaccine must be of high efficacy and producing only mild or transient adverse effects an acceptable and benefit-risk contour. Various vaccine platforms depend upon adjuvants for inducing the T-cell response. Live attenuated vaccines are not recommended, as a delivery vector increases the risk of pathogen conversion to actual viral infection. Viral vectored vaccines are effective in inducing T-cell response but sometimes their efficiency is affected by cross-reactive immunity (antibodies that neutralize the antibodies mobilized by the vaccine). Nucleic acid based vaccines are also a successful candidate but they need specific delivery vehicles or adjuvants. In many countries, emergence of new cases and transmission of COVID-19 diseases is significantly declining whereas in some countries the number of new cases increase day-by-day. Approved vaccines must be suitable for persons of all ages, pregnant mother, lactating mother as well as immunocompromised person. To meet the need or demand of less wealthy countries, the funding committed through the COVAX program (COVID-19 Vaccine Global Access) and coalition for epidemic preparedness innovation (CEPI) unite rich and low- income countries to achieve rapid and fair access to the most effective COVID-19 vaccines. Since vaccines alone cannot combat the pandemic and assure its elimination, prevention strategies and social strategies will remain needed and such strategies will also help us to face future pandemics.

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Using MapMCDA Tool for the Spatial Epidemiology of Animal Rabies in Morocco: How to Improve the Rationality of a Qualitative Risk Assessment

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ABSTRACT

Objective
The objective behind this article is to better characterize spatial distribution of animal rabies in Morocco through qualitative risk assessment framework. In Morocco, the occurrence of the disease is neither clearly distributed nor complete. Therefore, risk assessment methods become strongly recommended to cope with distorted geographic patterns.

Methods
Based on data collection set from 168 counties, qualitative changes on spatial epidemiology of rabies were analysed by mapMCDA tool covering a period from 2004 to 2017 and including information on determinants of the geographic distribution of animal rabies in Morocco defined in previous work.

Results
To validate the risk assessment model, the results were compared to rabies cases reported during the study period. The clustering of the rabies risk estimates is decisive and highly reliable. A significant alignment was shown between the very high and high-risk estimates.

Conclusion
This study is the first attempt that has been made for using MapMCDA for rabies. For a normative process aiming to avoid subjectivity related to expert-opinions, authors suggest conducting initially a statistical multiple component analysis that will provide quantified estimates of risk factors. It would be an advisable decision-making tool that helps to design oriented surveillance and allows better referral of actions to control the disease.

Keywords
Animal rabies; Canine rabies; Spatial epidemiology; Qualitative risk assessment; MapMCDA; Veterinary science; Public health; Morocco.

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INTRODUCTION

Spatial epidemiology is the description and analysis of geographic distributions and developmental changes in disease risk or incidence with geographical information system and geospatial analysis.[1,2] Since the use of geographic analysis in characterizing the spread and possible causes of outbreaks of infectious diseases dating back to the 1800s,[1] progress has been made in many aspects of spatial epidemiology, including disease mapping,[3,4] risk assessment in relation to point or line sources,[5-7] geographical correlation studies,[8] and cluster detection and disease clustering.[9,10]

Some studies have reviewed the related literature on spatial epidemiology and summarized the progress in this research field. Advances in approaches to investigate local spatial variations in diseases have been analyzed, and developments in exposure modeling and mapping, enhanced study designs, and new methods of surveillance of large health databases were keys to understand the complex relationships of environment to health.[1] Since the impacts of landscape structure on epidemiological processes have been often neglected, a true integration of landscape ecology with epidemiology was considered to be fruitful.[2] Exposure assessment was improved with developing geospatial analysis techniques to enable the visualization of uncertainty and ensure that more meaningful inferences are made from data.[11] Spatial methods, including geocoding, distance calculations, residential mobility, spatial aggregation, clustering, spatial smoothing and interpolation, and spatial regression, were widely used in epidemiology.[12,13]

Statistical analysis of the relationships between these data could highlight correlations between environmental variables and epidemiological variables, thus making it possible to better understand and possibly quantify the modes of transmission of a pathogen according to environmental conditions. This approach to analyzing patterns of transmission constitutes landscape epidemiology.[14-16] Landscape epidemiology describes how the dynamics of populations of hosts, vectors and pathogens interact spatially in an environment that makes transmission possible.[15] In general, different types of factors are involved in the evolution of animal diseases, whether genetic, biological, but also environmental, climatic, or political, economic, demographic and societal factors.[17]

Moreover, considering spatially distributed factors, spatial epidemiology has provided a valuable modeling framework for investigating the dynamics mediating the transmission of emerging diseases.[18] With the growing popularity of these studies, spatial measurement errors were found to be ubiquitous threats to the validity of spatial epidemiological studies, and so the various mechanisms generating these errors should be revealed.[19] In the last 10-years, although several spatial decision support systems have been developed to facilitate data collection, analysis, and decision-making, standardization for functionality and system development and flexible interfaces, they all still need to be addressed. Moreover, real-time distributions of the causative agents and their vectors can be updated rapidly by connecting remotely-sensed environmental records with terrestrial-captured data.[20]

Broadly speaking, the various studies carried out on the determinants of rabies in Morocco; contribute to a better understanding of the influence of these factors on the transmission of rabies. The contribution of an analytical approach to describe the patterns of transmission of the rabies virus according to environmental characteristics is detailed from examples from a previous research work. Based on the hypothesis by which landscape characteristics can explain the spatial heterogeneity of transmission of the rabies virus in Morocco, we illustrate this approach with a study on the alignment between dog habitat, socio-economic environmental characteristics and epidemiological data. A multiple correspondence analysis (MCA) statistical analysis followed by a linear regression to set an ascending hierarchical classification has first highlighted the existence of four determinants of animal rabies in Morocco, which can be interpreted to be risk factors of the disease.[21]

The multivariate logistic regression analysis method was chosen because the variable to be explained was binary and the explanatory variables were quantitative and qualitative. Qualitative risk assessment and disease mapping have been in particular useful in data-scarce environments, often encountered in developing countries, with little available quantitative data on potential risk factors.[22-24]

Despite being subjective and with a tendency to overestimate the risk,[25] the qualitative risk assessment approach has proven to be transparent and efficient to estimate the likelihood of animal disease occurrence when limited data are available.[26] Over the last few decades, different methods for risk assessment and risk mapping have been widely used to support targeted and cost-effective animal disease surveillance.[27]

While considering that national veterinary services are severely understaffed and suffering limited resources to keep up the quality of the data, there has been ultimately very relevant effort to identify geographic clusters of the disease to better focus control on high-risk areas. The research hypothesis that emerges from this is: will the qualitative risk assessment analysis be able to reduce uncertainty in terms of disease knowledge, try to draw up a geographical distribution of the disease which approaches the field situation and give then oriented control actions of the disease?

This is the starting point in this research study on risk assessment methods of animal rabies. The specific objectives and emergent research questions are to: (1) understand the changes on spatial epidemiology of rabies by using the determinants of the disease; (2) identify the burst of areas at risk and explore new pathways of rabies control related to spatial epidemiology.

Data Sources

In order to study the spatial epidemiology of animal rabies in Morocco, a data set have been collected to cover a broad range of indicators of environment and human activities as well as to reflect field reality. Using qualitative risk assessment methodologies enabled an in-depth profiling of animal rabies to be made. These data are as follow:

- A data collection from national information system (SIPS) cover-

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ing the period from 2004 to 2017. A total of 1 346 animal rabies cases were recorded.
- A data set of 3 528 records on habitat, human, social and economic related to 168 counties from the Moroccan "*High commission of planning*" (Census records of 2014).
- Data on animal rabies risk factors highlighted in previous study taking into account several factors as geographical affiliation, socio-economics and demographic characteristics of the environment in which dogs evolve in Morocco.
- A shapefile data that represents the 1,542 counties of Morocco that we collected from the high commission of planning.

METHODOLOGY

The data were prepared for use by following the steps outlined in the methodological model figure: Importation of data in quantum geographic information system (QGIS), data analyse on QGIS and mapMCDA.

Importing Data into QGIS

We have started by importing the Excel table which contains the data of the various risk factors in addition to the cases of rabies in affected dogs and animals spread over 168 counties using the delimited text tool. Then, we have imported the shapefile of the counties of Morocco using the vector tool.

Data Processing on QGIS

The risk factors are represented geographically by points which constitute the centroid of the municipalities. In order to use the data found in the attribute table of this layer, we have joined the attributes by location with the shapefile of the municipalities to allow the visualization of each risk factor. However, while using these factors each one separately at the mapMCDA tool, we have applied the rasterization tool (vector to raster).

mapMCDA

MapMCDA is an estimation method currently using linear functions for scaling that helps producing risk factor weighted risk maps using expert knowledge. The typical use case is the lack of reliable data on disease outbreaks, but the information available on risk factors is well-known.

In order to design a stratified sampling or surveillance campaign, a preliminary risk map based on expert judgment is needed. This package (and method) provides a systematic and guided approach to constructing such maps.

The set includes a graphical user interface (Glossy) that assists in the treatment and the weighting of risk factors.

The mapMCDA package facilitates the weighting of several risk factors to produce an epidemiological risk map.

Nevertheless, the expertise of the user is crucial and it is expressed at three levels:

1. Choice of relevant risk factors.
2. For each factor, common risk scaling (e.g. between 0 and 100).
3. Two-by-two assessment of the risk factors relative importance.

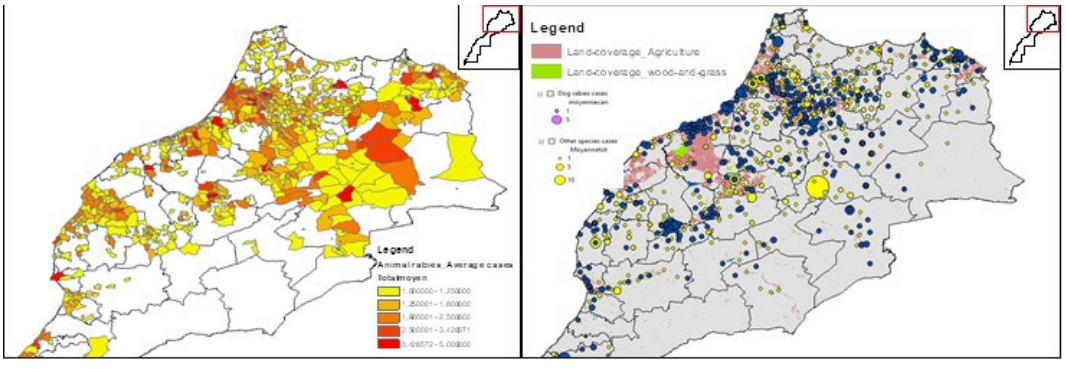
In accordance with the research design, methods described above are organized in terms of coverage and in-depth analysis. We first conducted a mapMCDA analysis, initially approached to characterize rabies spatial variability using qualitative risk assessment framework. We then applied a cross-validation using rabies data collection for a period from 2004 to 2017 to appreciate alignment meaning. Finally, we estimated the exposure effect to risk categories through rabies occurrence risk relative.

RESEARCH RESULTS

Analysis of Rabies Spatial Evolution in Morocco

Overall, the geographic distribution of animal rabies does not seem to have any particular shape because it does not only affect most of the provinces but its incidence at the provincial level also varies from year to year. However, it should be noted that the geographic pattern of rabies has changed significantly over the past 20-years. The rural environment accounts for the majority of the cases of animal rabies declared with 81% of the cases against 19% of the cases in the urban environment, thus testifying the imposing rural predominance of the disease (Figure 1).

Figure 1. *Rabies Geographic Distribution–Period: 2004-2017*



Furthermore, another important determinant of the spatial heterogeneity of the transmission of dog mediated rabies, listed in another research work, concerns aspects related to municipal infrastructure. Indeed, the existence of local veterinary services, municipal hygiene offices, dog poundages, fenced and controlled landfills as well as slaughterhouses is a must for waste management of carcass seizures. Hence, this will make it possible to avoid dog and animal rabies in this well-endowed geography, and in particular to contribute to better understand the determinants that transmit the rabies virus. In rural areas, slaughterhouses and livestock markets have not been under strict enforcement of regulations requiring policies for proper waste management, and are thus ideal gathering places for dogs. Therefore, elected spots of major contamination for free roaming dogs as well as for other animal species are subsequently developed in these areas. It appears that throwing away seizures of vicera and carcasses from these establishments into the wild without denaturing or incineration, or catering waste within livestock markets, constitute feed resources of choice for ownerless and stray dogs. This provides them with conditions of survival as well as good reproductive health. This context promotes then an excellent dynamic survival of the canine population and thus enhancing the transmission risk of rabies virus within and between animal species in areas with these determining factors (rural habitat, lower county development index (CDI), livestock markets, rural slaughterhouse, etc).[21]

Epidemiology of Rabies Using mapMCDA Tools

The mapMCDA tool uses as source data type: Vector, Raster and Network (CSV) (Figure 2).

Figure 2. *Data Types for mapMCDA*

```
known_extensions <- list(
  vector  = c("\\.gpkg$", "\\.shp$"),
  raster  = c("\\.tif$", "\\.tiff$", "\\.grd$", "\\.gri"),
  network = c("\\.csv$")
)
```

The risk factors on which this study is based are the four ones mentioned above (rural habitat, lower CDI, livestock markets, rural slaughterhouse).[21] We have compared their relative importance 2-by-2 on a scale of 0 to 9 and represented these relationships in a matrix which must have 1 in its diagonal. The system calculates the most consistent weights with these pairwise valuations, with the function "compute_weights". In this step, weights are assigned according to the impact of each factor which is precisely related to the OR calculated in previous research work (Table 1). In such way, the factor of row "i" is x [i,j] times greater than the factor of column "j".

For this study comma-separated values (CSV) tables will not be used mainly because there is no research interest for this study on dog mobility data, but also due to the shapefile format created which takes only into account the geometry and cannot specify which column of the attribute table would be considered as a risk factor (Table 2).

Table 1. *Results of the Modeling of Risk Factors Associated with Canine Rabies [Khayli et al[21]]*

	ORMac:	p	OR	p
Weekly Rural Markers	1.95	0.001	7.50	0.006
Rural Habitat	1.92	<0001	9.07	0.003
Human Density	2.05	0.003	8.76	0.003
Slaughter House	1.92	0.01	2.58	0.04

Table 2. *Risk Factors Included in mapMCDA Interface*

	Name_orig	Name_New	Type	Admin_unit
1	Counties_2015	communes_2015	Vector	☑
2	Density_CDI	Human Density/CDI	Raster	☐
3	Habitat_rur	Rural Habitat	Raster	☐
4	esslghthouserur	Rural slaughterhouse	Raster	☐
5	Ess_live_stock_market	Rural livestock market	Raster	☐

To convert data into usable risk factors on mapMCDA, each risk factor was represented in the form of a choropleth map, and then we rasterized (vector to raster).

Moreover, the layer we want to use as base vector layer was cheked in this layer. A column of the mapMCDA risk, obtained by combining the different risk factors is included in its attribute table.

In this step, the tool harmonizes original scales into a common risk scale between 0 and 100, to subsequently combine different factors using a specific weight.

Results provided by the package are series of risk maps based on risk factors weighting method (Figure 3). This analysis methods combination is able to fully reveal the influence of animal rabies distribution on veterinarian's ability to effectively implement enforcement activities against the disease in Morocco. For our study area, we model data with a risk map and here is what it looks like in Figure 4.

Validation step of mapMCDA findings requires the use of the rabies cases notified during the period 2004-2017. The substantiation process iterates through all the output areas generated by the mapMCDA tool (Figure 5).

Then, it creates a summary graph presenting the relationship between rabies cases locations and corresponding risk levels (Figure 6).

This figure shows how robust our quantitative risk assessment model really is and how close are identified areas to the real context. In other words, it expresses how well the model fits the data.

Interpretation

The rabies spatial distribution appears pointless and unclear given

Figure 3. *Weighing Risk Factors in mapMCDA Interface*

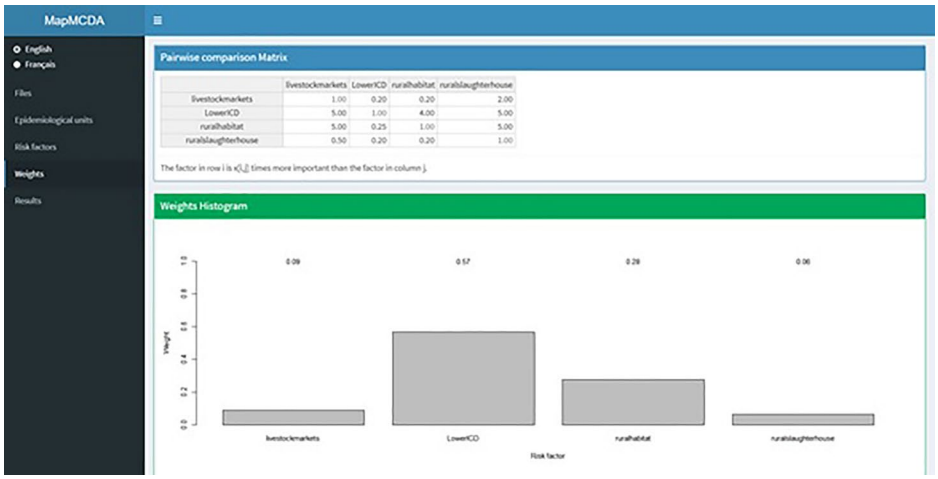
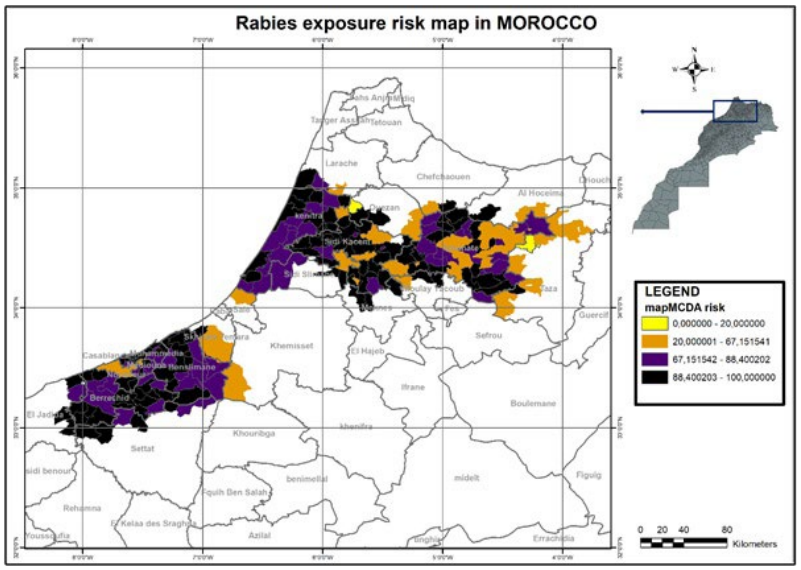


Figure 4. *mapMCDA Risk Map of Animal Rabies*



probably the underreporting of rabies cases. Spatial analysis does not allow concluding definitively on rabies hotspot locations. Nevertheless, a risk assessment analysis over a long period contributes to better understand involved processes and highlights importance of socio-economic factors, in addition to dog habitat determinant. This has been carried out in order to explain the rabies geographic distribution.

MapMCDA analysis over a period spread of 13-years allows establishing risk maps with more precision of the disease spatial distribution, in particular with the disease geographic clusters identified as well as validation process. At a glance, one of study results reveals the identification of animal rabies clusters in this environment which are dependent on the factors mentioned above. Results obtained of the disease geographic patterns during the study period show a clear discrimination of cases.

From this observation, this allows us to assess the impact of these factors, in terms of rabies maintenance in areas that were not previously a stronghold of the disease. It seems obvious that these risk areas can take over in terms of contamination and constitute a source for the spread of rabies throught the country.

As mentioned previously, the outcome in the model validation was the number of rabies cases in each risk area. This illustrated empirically a good alignment between the evaluation of risk estimates and the field situation.[26] To put all this in perspective, we have checked the proportion of area and rabies cases in each level of exposition, as it should be with a close significant association for very high and high. In addition, the number of rabies cases notified in low and negligible levels is shown to be so small. Out of 168 counties involved in this study, 32.21% were at very high-risk, 16.52% at high-risk, 15.44% at low risk and 24.85% at negligible

Figure 5. *Validation of mapMCDA Risk Map with Rabies Reported Cases*

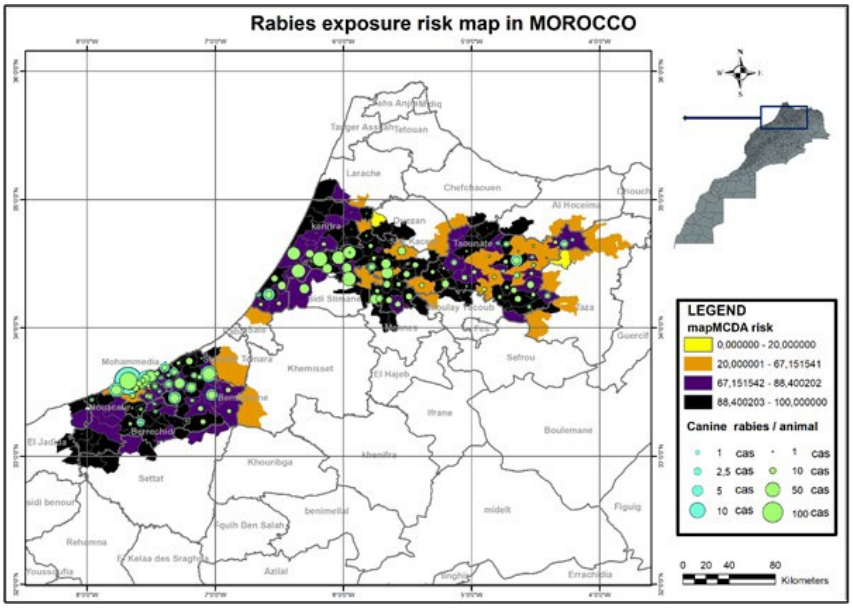
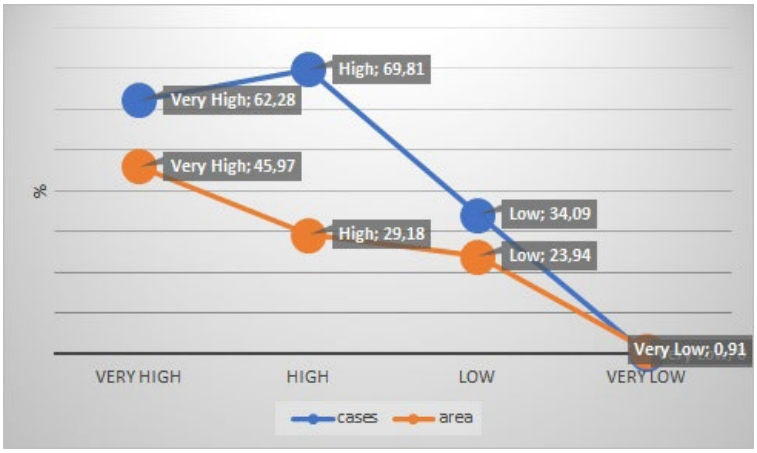


Figure 6. *Proportion of Area and Rabies Cases in each Level of Exposure*



risk of rabies exposure (Figure 5).

Clustering of rabies risk estimates is decisive and highly reliable; hotspots areas are related to epidemiological data. Very high and high-level risk have been validated by a proportion of 62.28% and 69.81% rabies cases, respectively (Figure 5). Using a Mantel-Haenszel test, a significant alignment between the very high and high-risk estimates was demonstrated. The risk relative estimated for very high and high-level was 3.01 higher than very lower and lower-level (95 % credible interval: [1.90-4.79], p value< 0.0000001), and thus is closely connected to field situation.

DISCUSSION

This article reports on risk assessment study considering the potential of reflective practice in animal rabies control and its impact on exploring new control approaches. Findings suggest that the understanding of animal rabies distribution in Morocco, as inferred from data analysis relates initialy to geographic patterns identification.

Specific biological, ecologic, environmental, and societal factors have been identified that precede emerging infections. Improved understanding and assessment of the complex factors associated with disease emergence and spread will lead to better management and thus reduction of risk for disease occurrence.[23] In fact, the application of a statistical model obtained by a linear regression shows that these models have a high capacity for discrimination but cannot be used for predictive purposes in new geographic areas except in a qualitative way (in terms of relative risk).[28] Therefore, coupling the results of these analyses with the results of spatial analyses makes it possible to determine whether

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an identified spatial aggregate results from greater transmission due to specific environmental conditions, or simply reveals greater vulnerability of populations (socio-economical conditions) at this location.[29]

Being able to review a larger number of risks and possible risk management strategies in one analysis gives the risk manager a better aerial view of the problem, and helps strategize at a more global level.[30] Nonetheless, all forms of risk assessment require the greatest possible collection and evaluation of data available on the risk issue, and require indepth knowledge in a variety of scientific disciplines.

Risk assessment can be either quantitative, (i.e., providing a numeric estimate of the probability of risk and the magnitude of the consequences), or qualitative, using a descriptive approach basing its assessment on the opinions of scientific panels.[31] Qualitative risk assessment has been used with great success in various arenas of project and military risk for over a decade, and has found interest in animal health-related areas.[30] Owing to the lack of relevant data and the very short period of time usually allowed to assess animal health risks on particular topics, this panel has been using a qualitative risk method for evaluating animal health risks or crises for the past few years.[31] In the absence of data, qualitative risk assessment frameworks have proved useful to assess risks associated with animal health diseases.[22]

Squarzoni-Diaw et al,[26] claimed that expert elicitation is crucial to identify the relevant risk factors for each risk pathway in the framework, but the method reveals possible uncertainty by the subjectivity of experts or the quality of data. In fact, some mathematical properties of risk matrices show that they have the following limitations: They can assign identical ratings to quantitatively very different risks, or can mistakingly assign higher qualitative ratings to quantitatively smaller risks. Inputs to risk matrices and resulting outputs (i.e., risk ratings) require subjective interpretation, and different users may obtain opposite ratings of the same quantitative risks.[25] For these reasons, little research rigorously validates their performance in actually improving risk management decisions, and some limitations suggest that risk matrices should be used with caution, and only with careful explanations of embedded judgments.[25] This approach has flaws and better methods are needed.[27] Nevertheless, scope exists to elaborate the current standards and guidance, which better serve the principle of science-based decision-making.[27]

In a previous study on animal rabies in Morocco, the environmental and anthropogenic data have been included as explanatory variables in the analysis related to dog habitat (urban or rural), level of municipal and community equipments (CDI) in terms of public health management infrastructures: pond or landfill, slaughterhouse, livestock market.[21] This condition was verified upstream of the statistical analysis (by verifying the independence of the variable to be explained by statistics measuring spatial dependence).[32]

Based on a MCA statistical analysis related to the study mentioned above, risk factors guidance was established. The rabies determinant's factor odd ratio (OR) have been already estimated in Morocco and a classification for weighing and evaluating risks with a score, allows to have an objective position and to avoid expert-opinion subjectivity. The original feature of this approach lies in the combination of epidemiological data and risk factors within the same framework. The graphic representation of the processed data is a series of hotspots of the study areas. Rabies determinants have been assessed for specific risk questions. The resulting hierarchical risk estimates have been determined by authors based on existing data and not through expert opinion elicitation.

The advantage of looking to risk assessment is that its large body of research has been conducted primarily on livestock animal diseases. Its ideas are based not on intuition, but on systematic observations and empirically supported conclusions that have withstood rigorous scientific testing. Our risk assessment study, as described here, can be included in both qualitative and quantitative rationale, but this may understate the important differences between both methods in their structure and their relative levels of objectivity. An Food and Agriculture Organization (FAO) report specific to semi-quantitative risk characterization claimed that it demands as prerequisites some statistical skills (e.g. multiple correspondence factor analysis) as quantitative risk assessment but it does not require the same amount of data; which means it can be applied to risks and strategies where precise data are missing. The integration of analytical epidemiology to obtain risk estimations opens a path for better analysis.

Authors describe a new set of levels and give more precise enumeration of categories that cover broad ranges of probability as well as the items considered when addressing animal health consequences.[31] This research study offers an improved level for the textual evaluation of qualitative risk assessment since it gives more consistent and rigorous approach to assessing and comparing risks than does conventional qualitative risk assessment. Using mapMCDA method offers sound evidence that can be applied toward increasing sharp analysis. So, then it avoids some ambiguities and will be able to reduce considerably the uncertainty.

For a normative process aiming to enhance objectivity of mapMCDA analysis, we suggest to conduct initially a statistical multiple correspondence analysis that will provide quantified estimates of risk factors. The obtained scores through OR or RR will be the key elements for weighing risk factors. This will be a fundamental step to avoid subjectivity of the expert-opinions on the spatial trends and to increase accuracy of the risk assessment analysis.

Through this risk assessment model, simulation of different epidemic geographic patterns of animal rabies shows the importance that certain municipalities influence the magnitude and severity of the epidemic. This illustrates empirically a good alignment between the evaluation of risk estimates and the field situation[26] and suggests that an oriented control on these areas would prevent large epidemics of rabies to spread at a large scale.

LIMITATIONS

This study may contribute to a better understanding of animal

rabies spatial distribution of in Morocco, and in particular, the importance of rabies and associated environmental variables. It also highlights limits of an analysis approach based on incomplete epidemiological data. Proportion of risk estimates areas that could not be validated by rabies occurrence is essentially due to unreported rabies cases. This highlights that the existing knowledge still not sufficient to fully understand field situation and can only give some cross-understanding of the disease.

As we evolve in contexts where data are severely missing- which could be considered as a limitation for this study, it is therefore imperative to explore and develop decision support tools adapted to rabies. Interpolative methods might be of a great benefit to build reliable spatial data of the disease and lead to implement robust risk-based control approach. The main purpose is to bring strength evidence on rabies geographic distribution to advocate for resources in line with the thoughts of risk-based approach and alternative cost-effective control possibilities.

CONCLUSION

The aim of this paper was to describe the improved risk assessment method taking into account the limitations of the conventional version. In conclusion, it can be said that mapMCDA is valued and is seen as an interesting qualitative risk assessment tool analysis when it addresses needs to identify rabies clusters. However, main rabies control challenge may lie in the dynamic interaction between new epidemiological analysis opportunities and service delivery requirements, as there maybe occasions where they vie with each other for resources.

Effectiveness of dog rabies control is inextricably linked to any improvements in required rabies data collection quality for epidemiological analysis. Use of risk assessment method such as mapMCDA would be more appropriate to correct gaps in epidemiological recording systems.

Development of new decision support tools, such as interpolation and prediction methods, seems to be decisive to exhibit robust spatial risk assessment of the disease, to target surveillance and finally to better streamline rabies control.

CONTRIBUTION

All authors have approved the final article. The data that support the findings of this study are available from the vet services of Morocco upon reasonable request.

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AUTHOR CONTRIBUTION STATEMENT

Khayli M and Kechna M conceived, designed the research study, analyzed and interpreted the data. All authors contributed materials analysis tools or data. Khayli M, Kichou F, Berrada J and Bouslikhan M wrote the paper.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This is a scientific article with original research data.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Factors Affecting Access to E-Learning during the Coronavirus Disease 2019 Pandemic Among Rural-Based Pharmacy Students in Zambia: A Qualitative Study

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ABSTRACT

Background
The coronavirus disease 2019 (COVID-19) pandemic has negatively affected the education sector globally. This has resulted in learning institutions adopting e-learning techniques. E-learning implementation in higher education continues to gain prominence in both developed and developing countries. Most universities are exploring different ways of using information and communications technology (ICT). However, ICT remains a challenge more especially for students who come from rural areas.

Aim
This study was aimed at exploring the factors that affect access to e-learning among rural-based pharmacy students in Zambia.

Methods
A qualitative case study was conducted among ten (10) purposively sampled pharmacy students at the University of Zambia. The study participants were from the Manying, a district of North-Western Province, the Sinda district of Eastern Province, the Nalolo district of Western Province, the Chipili district of Luapula Province and the Mbala district of Northern Province. Semi-structured interviews were used to collect data from the respondents. Data were analyzed using the framework analysis. The sociodemographic characteristics indicate that ten (10) respondents were drawn from Zambia's five (5) provinces. Six qualitative themes were generated these included devices used for e-learning; the effectiveness of the devices; student performance; internet connectivity; and electrification of the houses. Key findings suggest that the most commonly used device was a smartphone, which posed challenges to effective learner participation in e-learning. Poor internet connectivity, non-electrification of students' houses, electricity outages, and costs-associated with internet use negatively affected students in accessing online learning and could adversely affect their academic activities and performance.

Conclusion
The COVID-19 pandemic has negatively affected access to e-learning among rural pharmacy students in Zambia. The implications of the challenges faced by the rural pharmacy students are that their academic activities and performance were negatively affected. Therefore, this posed a threat to the rights to universal access to education of the rural students who were mostly venerable.

Keywords
Academic performance; COVID-19; Coronavirus disease; E-Learning; Online learning; Pandemic; Pharmacy students.

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INTRODUCTION

The origin of coronavirus disease 2019 (COVID-19) from China is marked as one of the greatest challenges to public health in human history.[1,2] COVID-19 emerged from Wuhan City of China in 2019 and spread rapidly to other countries in 2020 causing the World Health Organization (WHO) to declare it a pandemic.[3,4] COVID-19 posed many negative impacts to many sectors across many nations including their educational systems.[5]

The COVID-19 pandemic has negatively affected educational systems worldwide, leading to the total physical closure of schools, universities, and colleges in many countries.[6] By mid-April 2020, approximately 1.7 billion learners were affected worldwide due to school closures in response to the pandemic. According to UNESCO monitoring, 191 countries implemented nationwide closures as well as local closures, impacting about 98.4% of the world's student population.[7]

In the wake of continuity education for universities and colleges, there was an implementation of e-learning.[8-10] E-learning implementation in higher education continues to gain prominence in both developed and developing countries, and while most universities in Information and Communication Technology (ICT)-rich environments are exploring different ways of using ICT and multimedia resources to enhance teaching and learning, the same cannot be said about ICT-challenged environments. The disparity in terms of access to e-learning in different geographical locations of the country and rural areas may be termed challenging in this context.[11-13] Nevertheless, the question of successful and sustainable e-learning implementation remains a challenge, particularly in ICT-challenged environments.[14,15]

Zambia, a country in the Center of the Southern African Region only had its first two cases reported on the 18th of March 2020.[4] This resulted in the Zambian government declaring that all schools, colleges and universities be closed with immediate effect on Friday 20 March 2020. Because of this, most students or learners were forced to stay home and continue with their education *via* online platforms. Despite this unfortunate situation, students were expected to learn with the use of Web 2.0 tools. Unfortunately, accessible computers, phones, laptops, and tablets either at home or school were not affordable by the majority of the students.[11] However, policymakers and significant partners had anticipatory hopes to see learning go in a different direction in Zambia during the COVID-19 crisis. The opportunities after the crisis lie in the fact that lecturers and students will learn new ICT skills and blended learning will be a new culture in the Zambian educational systems.

The coming of technology has impacted almost all areas of life including the education sector that has been witnessing a paradigm shift.[16] The shift was made due to restrictions imposed by the ministry of health as a result of the COVID-19 crisis, and nearly all higher learning institutions in Zambia had shifted to digital learning with immediate effect. The University of Zambia (UNZA) Senate also resolved that in this closure, learning would proceed through e-learning platforms like Zoom, Moodle, and Astria, among others. Subsequently, academic staff was requested to expeditiously secure learning support material for them to facilitate teaching and learning using the e-learning platforms. Similarly, students were also guided to make sure that they registered and got connected to the e-learning platforms to avoid missing out on learning. This experience is adopted in the design of current study as it factors in the involvement of students as part of the study sample so that they could share their experiences on how they accessed e-learning during the COVID-19 pandemic crisis, taking into consideration the bottlenecks associated with the rural environment.

The literature plays a very important role in giving rise to the need to understand what kind of electronic devices were used by the rural students when accessing e-learning in their geographical settings and establishing the effectiveness of these devices used during the COVID-19 pandemic. It can be argued that Zambia has a massive literature gap on the impact of the COVID-19 pandemic on the education system. Therefore, this study endeavours to fill that literature gap by profiling the e-learning experiences of rural students during the closure of schools.

Theoretical Perspective

This study was underpinned by the e-learning theory. The theory comprises three elements as eluded by Dabbagh[17] defined through a theory-based framework that relates instructional strategies, learning technologies,, and pedagogical models or constructs. Dabbagh's framework (2005) includes multiple dimensions, such as the way people learn (open/flexible way), with the learning strategy (collaboration, exploration, problem-solving) and also with technology deployed in the learning process. "*Its pedagogical model and cognitive models or theoretical constructs are derived from knowledge acquisition models or views about cognition and knowledge, which form the basis for e-learning theory. In other words, they are the mechanisms by which we link theory to practice*".[18] From a pedagogical point of view, these models are mechanisms that link e-learning theory to e-learning practice (Figure 1).[17] The pedagogical models in e-learning are open learning, distributed learning, learning communities, communities of practice, and knowledge building communities.

Instructional strategies assist learning, such as collaboration, articulation, reflection, and role-playing among others. Instructional strategies operationalize the pedagogical models, since strategies consist of general approaches to a learning model, in other words; instructional strategies are enablers to learning. The instructional strategy facet of e-learning theory shapes the current study as it explores the modes of e-learning delivery to rural students in Zambia.

E-learning concept refers to learning *via* electronic sources, providing interactive distance learning. Today the e-learning concept, apart from technology, includes learning strategies, learning methods, and lately is very much directed to the vast possibilities of content diffusion and connection. The technological aspect of the theory informs the research question on the type of devices used by rural students to access e-learning. Therefore, this study was conducted to explore the factors that affect access to e-learning during the COVID-19 pandemic among pharmacy students in Zambia.

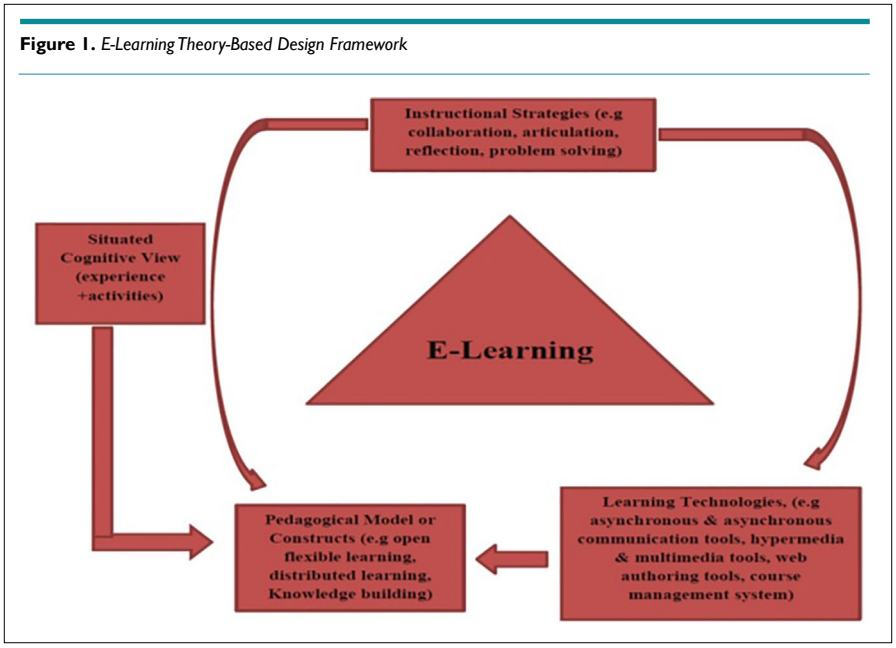


Figure 1. *E-Learning Theory-Based Design Framework*

Figure 1 indicates that the theory-based design framework for e-learning emphasizes the transformative interaction between pedagogical models, instructional strategies, and learning technologies.[17] It may be contended that situated or distributed cognition is an appropriate foundational knowledge perspective from which to develop pedagogical models and constructs for e-learning and offers a theory-into-practice framework that characterizes the instructional implications of situated cognition and guides the design of e-learning. This interaction is extensively explored in this study and how it ultimately affects academic performance for rural students.

METHODOLOGY

Study Design

This was a qualitative case study that was conducted among pharmacy students at the University of Zambia. This study was conducted amidst the COVID-19 pandemic that has disrupted physical classes in Zambia. This was a preliminary study of which a larger study will be conducted that will comprise of students from different programmes of study

Sample Size

The total sample size for the current study was ten (10) students who came from five (5) out of the ten (10) provinces of Zambia; two (2) students were picked from a rural district of the five (5) provinces. The respective rural districts were Manyinga of North-western Province, Sinda of Eastern Province, Nalolo of Western Province, Chipili of Luapula Province and Mbala of Northern Province. The districts met the researcher's desired core characteristic of being defined as rural. The students were all registered third-year Pharmacy students at the University of Zambia in 2021. The third-year pharmacy students were picked because during the physical closure of classes, they had remained to conduct their practicum. A sample size of ten (10) was arrived at because saturation was reached. Using a sample size of ten (10) in qualitative studies has been reported to be effective and enough provided the participants give more information relevant to the study.[19-21]

Sampling Technique

The respondents for the interviews were purposively sampled. Kombo and Tromp, 2006, suggest that in purposive sampling, the researcher targets a group of people believed to be reliable for the study.[22] The Snowball sampling technique was specifically used in this study. Snowball sampling is a recruitment method that employs research into participants' social networks to access specific populations. One student who comes from a rural area was identified, who then, in turn, identified other students from rural districts of different provinces of Zambia.

Data Collection

The semi-structured face to face interviews were used to collect data from the respondents. The method is advantageous because it allows direct questions to respondents about their activities. Semi-structured interviews were opted because of their flexibility in that they allow more specific issues to be addressed, elicit interpretations from the respondents, follow-up on the points that were not clear in the narrations of the respondents were made and probing where necessary. The interview guide also helped the researcher to be more systematic and to keep track of the goals of the study. The participants were chosen because they were available during school closures and the interviews were conducted strictly adhering to the COVID-19 preventive measures.

Data Analysis

Framework analysis was used to analyze the data. In the analysis stage, the gathered data was sifted, charted and sorted per key issues and themes. The themes were identified and drawn from the

responses to the questions given by the respondents. The framework approach offers the researcher a systematic structure to manage, analyze and identify themes, enabling the development and maintenance of a transparent audit trail. It is particularly useful with large volumes of text and is suitable for use with different qualitative approaches. Ward et al[23] eludes that the Framework analysis is flexible during the analysis process in that it allows the user to either collect all the data and then analyze it or do data analysis during the collection process.

RESULTS

The sociodemographic characteristics (Table 1) indicate that the ten (10) respondents were selected from the five (5) out of ten (10) provinces of Zambia. The respective rural districts were Manyinga of Northwestern Province, Sinda of Eastern province, Nalolo of Western province, Chipili of Luapula province and Mbala of Northern province. The students were all third-year Pharmacy students at the University of Zambia as shown in Table 1.

Table 1. *Sociodemographic Characteristics of the Respondents*

Province	District	Program of Study	Number of Respondents
Northwestern	Manyinga	Bachelor of Pharmacy	2
Eastern	Sinda	Bachelor of Pharmacy	2
Western	Nalolo	Bachelor of pharmacy	2
Luapula	Chipili	Bachelor of Pharmacy	2
Northern	Mbala	Bachelor of Pharmacy	2

Figure 2 shows that there was a male predominance of 6 (60%) in this study.

The effects of COVID-19 on the academic performance of the respondents are given in Table 2. Assessment 1 was done before closure of the university while assessment 2 was done during closure of the university. As indicated, many about 90% of the students had their academic performance based on the assessments they wrote during the COVID-19 pandemic in 2020.

Figure 2. *Gender of Respondents*

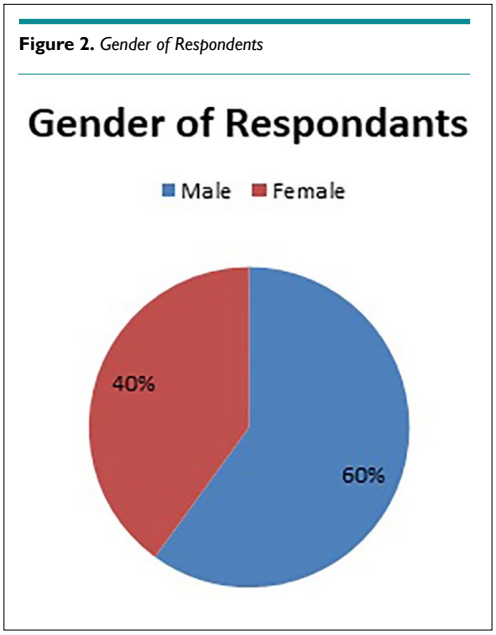
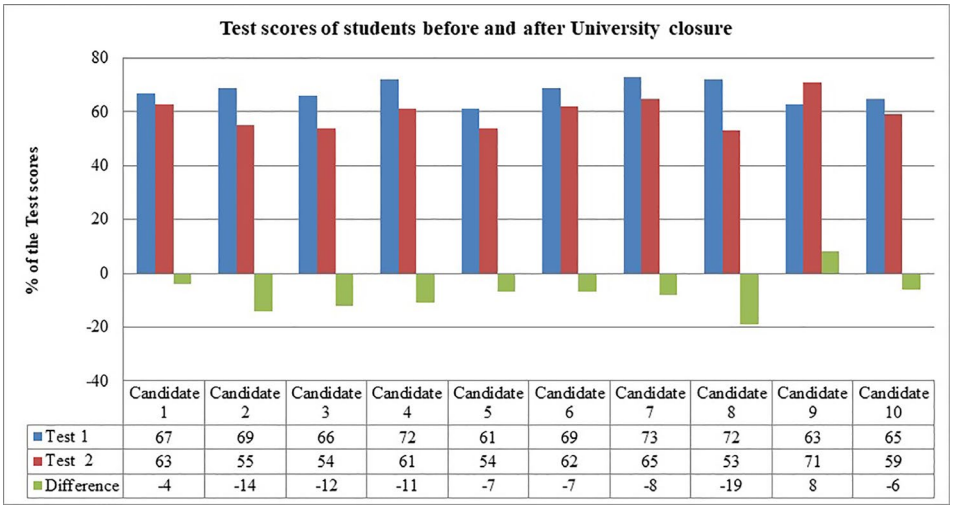


Table 2 shows responses from the participants regarding factors that affect their e-learning during the COVID-19 pandemic. The results have been presented in six (6) different themes.

Figure 3 Effect of COVID-19 on academic performance of rural pharmacy students.

The Figure 3 indicates the document analysis of the test results of the 10 students, test one was conducted before the closure of the university while test 2 was conducted online during the closure of the university due the COVID-19 pandemic. The test

Figure 3. *Effect of COVID-19 on Academic Performance of Rural Pharmacy Students*



	Candidate 1	Candidate 2	Candidate 3	Candidate 4	Candidate 5	Candidate 6	Candidate 7	Candidate 8	Candidate 9	Candidate 10
Test 1	67	69	66	72	61	69	73	72	63	65
Test 2	63	55	54	61	54	62	65	53	71	59
Difference	-4	-14	-12	-11	-7	-7	-8	-19	8	-6

Table 2. *Framework Analysis Table*

Theme	North-Western Province	Eastern Province	Western Province	Luapula Province	Northern -Province
	Manyinga District	**Sinda District**	**Nalolo District**	**Chipili District**	**Mbala District**
Electronic device used for e-learning	• Both respondents indicated that he relied on the phone to attend online lessons.	• One student depended on a mobile phone and a laptop to access e-learning. • The other student entirely relied on the phone as he had no laptop.	• One of the students used the phone and laptop to access e-learning, while the other one relied on the mobile phone only. • "*I was using a mobile phone, and sometimes the laptop but I mostly had challenges with the laptop*".	• The students used mobile phones to access learning. • "*I was using an android phone that I bought in 2017 when I was in Secondary School, it is an old phone that sometimes switches off on its own, and right now as I am speaking I have left it charging*". • The students did not have laptops because they were unaffordable.	• Both students depended on mobile phone to access e-learning. • One of the students stated that he could not use his laptop because he lived in a non-electrified house and his laptop could only sustain power for two hours.
Effectiveness of the device	• The respondents described the phone as not being effective in attending an online lesson. • "*my phone had issues with the battery, I needed to charge it every after an hour*".	• The students linked the effectiveness of the phone and laptop to the applications that were used to access e-learning. • Some applications were not user friendly to the students. They highlighted some of the applications used as Zoom, Moodle and Google meet.	• One of the students rated the effectiveness of the phone as 5 out of 10. • "*In terms of displaying the information the screen of the mobile phone is not large enough. And when holding the phone you would find that your neck would pain after holding for a long*".	• The phone was not effective as it was old and could not support some applications.	• The students described the tools as not very effective due to poor connectivity.
Internet connectivity	• The respondents indicated that internet connectivity was a challenge. • "*The network was sometimes unstable during class; hence I would be cut off.*"	• One student cited internet connectivity as a challenge in e-learning. • "*Yes I remember at some point there was a time when we had difficulties with the three internet service network providers, and this was national wide*". • The students missed some online lectures due to poor internet connectivity. "*This usually happened where I could be cut off because of poor internet connectivity*".	• She described internet connectivity as a big challenge as it was most unstable. • When asked how she felt when she was cut off due to connectivity. • I used to feel bad was just praying that we open schools soon because my performance went down. It made me disturbed because you would find that you don't know what the lecturer talk about when you were cut off.	• Both students described internet connectivity as unstable. • Asked to recall any day when there was poor connectivity. One of the students stated; • "*yes my very bad experience was when I was writing pharmaceutical chemistry test and just after answering 20 questions out of 33 my internet was disrupted, meaning that I had left 13 questions unanswered*".	• Internet connectivity was very unstable. • Asked to recall any bad experiences they had with internet connectivity one student said; • "*You would find that you are writing a test and the page is reloading and before you even finish it has submitted for you. Sometimes the page is not moving to the next page. Sometimes the page would not change but time is moving, and the system would submit when the time has elapsed*".
Cost of e-learning	• One of the students described the cost of e-learning as fair. • This suggests that the student did not face many adverse financial challenges to access the e-learning.	• Both students indicated that the cost of buying internet bundles was high. • One of the students indicated that the cost of the laptop and the phone was not a big problem. • "*I think with the devices it was not so much of an issue because I already had these devices before the pandemic*".	• The respondents indicated that e-learning was expensive as they were expected to always have money to buy bundles for the internet.	• Subject lamented about the cost of internet data bundles.	• The students indicated that e-learning needed high-quality electronic devices that could connect to a 4G network which the subject could not afford.
Student performance	• The performance of both students during online learning went down compared to the performance during on-site learning. • "*My performance on campus was much better than when I was home, for example at home there are no facilities such as the library*". • This indicates that the students were not able to have access to a conducive environment for studying.	• Both students from Sinda district indicated that their performance was much better with the physical learning on campus than with e-learning. • Suggest minimal student lecture interaction during e-learning as a factor that contributed to poor performance by the students. • The other factor highlighted by one of the students was house chores, as a hindrance to smooth learning.	• The students lamented that their performance was drastically affected by e-learning, "*my performance went down because when we just opened we were expected to have physical tests and my performance was really bad*".	• Both students described their academic performance as bad during the period of e-learning. • "*I felt frustrated; this was because my performance had drastically gone down. I was not performing as well as I was doing during the time as was in school. What was hurting more was that I am in my third year and I am expected to make points for my graduation grade*".	• The students reported that that they performed better with physical onsite learning than the e-learning. • Asked if they were exempted from house chores? one of the students replied that responded that "*It was difficult for parents to understand how e-learning works because it was quite new, sometime they would think you would not just want to help but probably you would just want to play with the laptop*".

Electrification of the house	• Both students lived in electrified houses but faced challenges of power cuts because the electricity power supply company undertook load shedding. • This suggests that the devices that the students depended on for e-learning were sometimes off due to loadshedding (electricity outages).	• Both students were living in electrified houses but also complained about the issue of power outages.	• The students were living in houses that were not electrified "*My house was not electrified so I used to go to my friend's house that was electrified, however, I could not go there anytime I had to make sure she was home it was her parent's home*".	• Their houses were not electrified and this adversely affected their studies.	• Both students lived in non-electrified houses. • This negatively affected the students as they needed to take their mobile phones for charging at the neighbourhood at a fee.

Figure 4. *Mapping of e-learning Experiences*

scores for 9 out of 10 students show a reduction at varying degrees this suggest that students performed better during physical learning than online learning.

The Figure 4 summarizes the e-learning experiences of the students sampled for the five (5) provinces, it is noted that the students faced similar challenges at varying degrees. Difficulties faced by the students ultimately negatively affected their academic performance.

DISCUSSION

E-learning has been embraced by nearly all teaching institutions globally, including Zambia during the COVID-19 pandemic.[24] The process of e-learning rests on several factors including the quality of the internet and speed, ease accessibility to online resources, the availability of suitable learning infrastructures along the readiness of both lecturers and students to adjust to this technology.[25,26] In the current study, we explored the factors that affect access to e-learning during the COVID-19 pandemic among pharmacy students in Zambia.

Devices Used for e-Learning

The findings from the generated themes suggest that students coming from different rural provincial areas faced very similar difficulties in accessing e-learning. It was established that all the students depended on their mobile phones as the main device for their e-learning. This finding is also highlighted by a study that was conducted in Ghana, which reported that distance learning students find it easier to use a smartphone in their learning activities.[27] Six of the students did not have laptops except for the students coming from western and eastern provinces, what that meant was that the students without laptops could not adequately do some assignments on their own, but had to take them for typing which affected the quality of their work. This concern raised by the participants in this study was also cited by educators in the United States of America (USA) after the lockdown due to COVID-19. Educa-

tors were worried that not all children have laptops or the Internet in their homes.[28] The lack of computers/laptops and computer labs was reported in another study as one of the factors causing challenges in students accessing online learning.[9] Additionally, in a descriptive cross-sectional study conducted at Liaquat College of Medicine and Dentistry. The students preferred using mobile phones to laptops during e-learning.[29] This could be because of increased teacher-student interactions and the improvement of communication in the classroom when using mobile phones than other devices.[30] Roberts et al[31] also established in their study that students preferred using mobile phones during e-learning.

Effectiveness of the e-Devices

The availability of appropriate and adequate technology is the mainstay of the ultimate delivery of e-learning. Technology helps in improving the quality of the e-learning experience through content integration and communication of learning material content at reliable speed and acceptable response times especially in a remote e-learning setup.[32] It was established that all the students from the five provinces described their tools as not being very effective at varying degrees, one student from the Eastern province had a better phone and laptop to access e-learning than the rest of the students whilst the student from Luapula province had the worst phone that was bought four years ago. His phone proved to be unreliable, such that it could switch off on its own during online classes. The two students from Western and Eastern provinces who had laptops both described them as not effective because they needed much stable internet connectivity and more data bundles compared to the phones. Studies have reported challenges faced during e-learning such as poor internet, unreliable phones and interruptions during teaching.[33-35] Considering the ineffectiveness of the devices used by the students in accessing e-learning, the researchers infer that the conditions in the rural districts of Zambia are not favourable to support e-learning.

Cost of e-Learning

Eight (8) out of the ten (10) students described e-learning as a financial burden. The students from Eastern Province were relatively financially stable compared to the rest of the students. The students associated the costs to mainly their inability to buy good Android operating system based smartphones and laptops. The cost was also mainly incurred on buying internet data bundles. The most affected student was a female student from the Western Province who despite having the laptop missed 75% of her classes mainly because of poor connectivity and lack of money to buy internet data bundles. The high cost of learning associated with e-learning is in concord with the report by Adeoye et al.[36] Despite this unfortunate situation of the closure of schools due to the COVID-19 pandemic, students were expected to learn with the use of web 2.0 tools. Unfortunately, accessible computers, smartphones, laptops and tablets either at home or school were not affordable by the majority of the students. Similarly, the use of online learning platforms comes with huge costs more especially to university students.[37] A study by Mahdy on the impact of COVID-19 on the academic performance of students established that availability, speed and cost of the internet were some of the common problems associated with e-learning especially for students who live in provincial and rural areas as these can hinder proper delivery of study materials by both students and lecturers.[38] Another study that was conducted in Jordan cited the poor economic conditions as challenges faced by students from the rural and remote areas during e-learning amid COVID-19.[39] Accessibility to online learning is affected by the cost attached to it.[4] Therefore, there is a need to consider the cost associated with attending online learning by University students.

Internet Connectivity

All of the ten (10) participating respondent student described the internet connectivity as unstable at varying degrees; the most affected student was from the Western province that had to ride a bicycle for 65 Km to the nearest place that had at least stable internet connectivity. The poor connectivity adversely affected students as they had to miss most of the lectures due to instability. This finding is in line with the assertion by Awidi[14] that successful and sustainable e-learning implementation remains a challenge, particularly in ICT-challenged environments. Internet challenges make it difficult to conduct and attend online learning.[9,14] It was notable that students from rural districts of Zambia were faced with the critical challenge of poor internet connectivity. Poor internet connectivity is a hindrance to quality education among students coming from rural areas.[34,40] Another study that was conducted in South Africa highlighted similar findings where students in rural areas were faced with challenges of internet connectivity during online learning amid the COVID-19 pandemic.[41] It is important to note that adequate infrastructures capable of supporting a successful deployment of e-learning based projects are needed. That is good internet connectivity and proper devices needed for sharing learning content with potential learners.[32]

Non-Electrification of Student's Houses

All the students except two from Eastern province were living in in home had no electricity it presenting difficulties in charging their laptops and mobile phones. This caused them to miss lectures because they sometimes had to take their mobile phones for charging during online classes. Further, the lack of electrification of the students' houses also affected their ability to study, especially at night. According to Awidi[14] he described e-learning is the use of ICT devices, Internet and World Wide Web resources, as instruments to construct knowledge, to support teaching and learning in synchronous and/or asynchronous modes. Non-electrification of student's houses hinders them from accessing e-learning.[4] Similarly, a lack of electrification and power outages in some areas negatively affects students' online learning and has been cited as among the inequalities affecting access to education.[42] The findings suggest that asynchronous modes of learning where online lessons can be recorded and students watch at their own convenient time should be explored to abate the challenges associated with lack of electrification and poor internet connectivity.

Academic Performance

All the ten students interviewed indicated that their academic per-

formance had decreased during the e-learning period and this was attributed to some factors such as divided attention between house chores and online class time, poor internet connectivity, lack of electrification of their houses, and the high cost of internet data bundles. Though the assessments used in this study indicated that nine students had their academic performance reduced during as a result of the effect of COVID-19. It was, therefore, notable that e-learning, if not cautiously implemented may widen the gap in terms of access to education between the rural and urban students. Similar findings have been reported by other scholars. In a study by Sintema, it was reported that the academic performance of students reduced as a result of COVID-19 and the introduction of e-learning.[43] Similarly, a recent study among pharmacy students indicated that the students were worried about their academic performance as online learning was the first time to them and the COVID-19 pandemic had disturbed them mentally.[44] In this regard, schools, colleges and universities must put in place measures to address the gaps that exist between rural and urban students in accessing online learning. On the other hand, one study reviewed that students thought that e-learning was better than traditional face to face learning as it provided convenience and portability as students can access it anywhere.[45] A cross-sectional study conducted by Mahdy[38] to analyze the impact of COVID-19 lockdown on the academic performance of veterinary medical students and researchers reviewed that most of the participants 96.7% believed that COVID-19 pandemic lockdown affected their academic performance with varying degrees. Despite the students' worries about academic performance, online learning must be embraced and implemented in the best way that supports continued learning.[46]

STRENGTHS AND LIMITATIONS

This study is important as it provides information on the challenges faced by rural pharmacy students at the University of Zambia and rural distance learning students engaged elsewhere. These challenges are not only limited to pharmacy students but all rural University students in Zambia. Therefore, these findings can help in developing strategies aimed at addressing the challenges faced by rural university students. The limitation to this study was that it was purely qualitative in nature; it did not use a randomized process to collect data, hence making it susceptible to biases. However, this limitation was abated somewhat in the selection of the participants.

CONCLUSION

It was established that the rural students largely depended on smartphones to access e-learning during the closure of schools as a result of the COVID-19 pandemic. Even when some students sometimes used the laptops, they described them as not being reliable because they demanded much more stable internet connectivity and constant power supply to access online lectures. Almost all the students described the smartphones and laptops as not being very effective in accessing e-learning, this was attributed to the low quality of the smartphones that they possessed such as inability to sustain power for a longer period, smaller screens to display legible PowerPoint slides and living in a non-electrified house. The respondents unanimously described e-learning as a huge cost as they were coming from vulnerable families, they all indicated that e-learning needed high-quality electronic devices and adequate data bundles which were not within their means. Key among the challenges faced by the students was poor internet connectivity in rural areas; some respondents had to walk long distances to the nearest place that had at least stable internet. The implications of the challenges faced by the rural students are that their academic performance was drastically negatively affected; therefore, this posed a threat to the rights to universal access to education of the rural students who were mostly venerable.

RECOMMENDATIONS OF THE STUDY

1. Learning institutions need to put in strategies to mitigate the challenges faced by vulnerable students during the COVID-19 pandemic that has negatively affected the education sector. Strategies may include provision of asynchronous learning so that students can be attending online classes at their own convenience.
2. Government should come up with initiatives to support vulnerable students with devices such as smartphones and laptops during the period of online learning.

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ETHICS STATEMENT

This study was approved by the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC). Protocol ID: 2020310174. IORG no: 0009227 IRB no: 00011000 FWA no: 00026270. After IRB ethical approval, regulatory approval was obtained from the National Health Research Authority (NHRA). Consent to participate in this study was obtained from the students.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Narrative Review

A Review of the Antiviral Activity of Ivermectin and Its Use in the Treatment of Coronavirus Disease-2019

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ABSTRACT

Background
The coronavirus disease-2019 (COVID-19) originated in China and was declared a pandemic by the World Health Organization (WHO) on 11th March 2020. Since its emergence in December 2019, there have been challenges in developing drugs that are effective against the virus. Currently, COVID-19 is managed using symptomatic and supportive therapies, antiviral agents, cellular and immunotherapy. Besides, most of the treatment modalities are still under investigation and treatment guidelines vary from one country to another. Ivermectin is among the drugs that are being used as part of treatment guidelines in certain countries like the Republic of Peru. However, the WHO recommends that ivermectin only be used in clinical trials.

Aim
The authors conducted this review to explore published studies on the possible therapeutic effects of ivermectin against active infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a causative agent of COVID-19.

Methods
A literature search was conducted using Google Scholar, PubMed and EMBASE for articles published from 2016 to 2021. Search words used included ivermectin, antiviral, COVID-19, efficacy, safety, dosing, lower mortality rate, hospitalised patients and the Boolean operator 'AND'.

Results
A few clinical trials have shown that ivermectin is safe for use in humans at specific doses and reduces the severity of the infection. Ivermectin was seen to reduce the signs and symptoms associated with COVID-19 in some studies while others showed no significant reduction. However, more studies must be conducted to ascertain its use in treating COVID-19.

Conclusion
Since many clinical trials are being conducted on the use of ivermectin to treat COVID-19, full evidence will be used to support its use in humans. Currently, some countries that are using ivermectin for treating COVID-19 have reported it to be effective and reduces morbidity and mortality associated with the disease. Therefore, countries should collaborate and provide full evidence for the use of ivermectin in humans to manage COVID-19.

Keywords
Ivermectin; COVID-19; SARS-CoV-2; Clinical trials; Antiviral.

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INTRODUCTION

The World Health Organization (WHO) received reports of pneumonia of unknown aetiology diagnosed in Wuhan, Hubei province of China on 31st December 2019.[1,2] In January 2020, the causative agent of pneumonia of unknown aetiology was identified as a novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the disease was later named coronavirus disease 2019 (COVID-19).[3,4] COVID-19 was eventually declared a global pandemic by the WHO on 11th March 2020.[5,6]

The signs and symptoms of COVID-19 vary depending on the severity and co-morbidities. The common signs and symptoms of COVID-19 are fever, dry cough, fatigue, sore throat, diarrhoea, headache, loss of taste and smell, dyspnoea, shortness of breath and chest pain or pressure.[7-13]

Different therapies are used for managing COVID-19 with variable treatment outcomes in patients. Antivirals, anticoagulants and anti-inflammatory agents that among agents used. Ivermectin is an antiviral agent and targets the host importin (IMP) α/β1 nuclear transport proteins responsible for nuclear entry of cargoes such as integrase and non-structural protein 5 (NS5)[14,15] and hence its use against COVID-19 which is being investigated in clinical trials.[16] In some countries such as the Republic of Peru and the North-eastern Beni region of Bolivia, ivermectin has been approved for treatment of COVID-19 in humans.[17] Notably, about 70 trials globally are presently testing the clinical benefit of ivermectin to treat or prevent SARS-CoV-2.[17,18]

METHODOS

Study Design

The literature used in this review was searched using Google scholar, PubMed and Excerpta Medica dataBASE (EMBASE). The keywords that were used in the search included ivermectin, COVID-19, antiviral activity, efficacy, safety, dosing, lower mortality rate, hospitalised patients and the Boolean operator 'AND'. This narrative review was performed from June 2021 to September 2021. We included all articles that were published in English from January 2016 to September 2021. We excluded all articles that had an abstract only. From a total of 65 articles that were retrieved, only 40 were used in our review based on the inclusion and exclusion criteria.

RESULTS AND DISCUSSION

Antiviral Activity of Ivermectin

Ivermectin is a Food and Drug Administration (FDA)-approved broad-spectrum anti-parasitic agent with demonstrated *in vitro* antiviral activity against some deoxyribonucleic acid (DNA)[19] and ribonucleic acid (RNA) viruses,[20] including SARS-CoV-2.[16,21-23]

The antiviral activity of Ivermectin is through the inhibition of nuclear import proteins of viruses as well as those of the host.[24,25] Ivermectin was originally noted to inhibit human immunodeficiency virus-1 (HIV-1) replication by inhibiting the interaction between the HIV-1 integrase protein and the importin (IMP) 1 heterodimer which is responsible for integrase protein nuclear import.[19,23] Ivermectin inhibits the transporter complex "*nuclear transporter mediated by a/β importin*", which is fundamental to the viral replication process; and binding to RNA-dependent RNA polymerases.[26,27] This prevents viral proteins from entering the nucleus, hence thereby reducing the inhibiting antiviral responses and leading to an efficient antiviral response.[20] Ivermectin also acts by inhibiting the nuclear import of *UL42* which is an accessory unit of DNA polymerase.[19,24]

Some studies done on SARS-CoV proteins have revealed that IMP α/β has a potential role during the infection in the signal-dependent nucleocytoplasmic shutting of the SARS-CoV nucleocapsid protein which may impact host cell division.[17,25,28] Most RNA viruses depend on IMP α/β for the transport of viral proteins at the time of infection, and ivermectin inhibits this import in thus enhancing antiviral activity observed in SARS-CoV-2 studies.[24] The transmembrane CD147 along with angiotensin-converting enzyme-2 (ACE-2) has been recognized as a major binding site for SARS-CoV-2 spike proteins.[24] Ivermectin has been shown to shield the spike proteins from the host receptor cells thereby interfering with the attachment of SARS-CoV-2 spike protein to the human cell membranes.[22,24]

It was proposed that human studies have to be conducted to fully observe the antiviral activity of Ivermectin earlier observed. Following-up, a pilot trial assessed the antiviral effects and safety of various doses of ivermectin in patients with mild symptoms of COVID-19. A total of 32 patients were enrolled who were randomly assigned to four groups that received standard of care treatment at hospital admission; standard of care treatment plus ivermectin 100 mcg/kg; standard of care treatment plus ivermectin 200 mcg/kg; or standard of care treatment plus ivermectin 400 mcg/kg.[29] In this study, the primary endpoint was testing negative twice for the SARS-CoV-2 reverse transcription–polymerase chain reaction (RT-PCR) test after 7-days of hospital admission. The results showed that those who received standard of care treatment plus ivermectin had a higher reduction in viral load compared to those who received standard of care treatment alone in a dose-dependent manner. This was also reported in a similar study by Pott-Junior et al.[16] This pilot study demonstrates that ivermectin combined with standard of care treatment in the management of COVID-19 presents an effective and adjuvant therapy. However, there is a need to conduct multi-centre studies to provide evidence that can be generalized to other patients.

Dosing of Ivermectin: Ivermectin Dose that Reaches the IC50 in the Lungs after Oral Administration

Ivermectin has a valuable clinical role in the management of different parasitic and helminthic diseases.[30] Ivermectin can be administered orally, subcutaneously, intramuscularly or topically at a dose range of 150-200 μg/kg in humans.[31] From some of the studies conducted, ivermectin has been reported to inhibit SARS-CoV-2 *in vitro* at certain concentrations. Caly et al[28] reported that Ivermectin inhibited SARS-CoV-2 *in vitro* causing a 5000-fold reduction in

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viral RNA at 48-hours with ivermectin at 5 μM. The concentration resulting in 50% inhibition (IC50) of 2 μM (1,750 ng/mL) is over 35 times higher than the maximum plasma concentration (Cmax) of 0.05 μM (46.6 ng/mL) after oral administration of the approved dose (~200 /kg μg) and ivermectin showed little to no activity at 1 μM *in vitro*. Because Ivermectin is highly bound to serum albumin (93%), the IC50 is in orders of magnitude higher than the unbound plasma Cmax after approved doses of ivermectin (0.0035 μM; 3.26 ng/mL).[20,28]

However, to translate the *in vitro* activity of ivermectin and relate it to the activity in humans, there is a need to evaluate these concentrations and compare them to the lung concentrations of orally administered Ivermectin.[32] For ivermectin to reach the lungs, all the pharmacokinetic parameters have to be considered as in theory, only the unbound drug reaches the lungs and other tissues through passive diffusion.[32,33] The likelihood of Ivermectin reaching the lungs after oral administration is related to its high lipophilicity, low ionization at physiological pH, protein binding capacity (about 93% serum albumin), as well as the transporters that maintain tissue distribution.[32]

Schmith et al[32] revealed that the total plasma concentration of ivermectin (bound and unbound) do not reach the IC50 as reported by Caly et al[28] even for a dose that is 10 times higher than the approved dose of ivermectin or after it is dosed repeatedly. The study showed that the plasma exposures did not increase substantially after repeat dosing with ivermectin accumulation in plasma after weekly dosing being very limited. The study showed that even with the high lung homogenate to plasma ratio, ivermectin is unlikely to reach the IC50 of 2 μM in the lungs after single oral administration of approved dose (predicted lung concentration being 0.0873 μM) or at doses 10 times higher than the approved dose after oral administration (predicted lung concentration being 0.820 μM). The study suggested that the approved dose of ivermectin alone has a low chance of being successful in the treatment of COVID-19.[32]

To reach the targeted concentration for the proposed mechanism of action for ivermectin, its IC50 is supposed to be 17 μmol/L.[14] Ivermectin antiviral concentrations expected to be effective are an overdose which could penetrate the blood-brain barrier and produce main adverse events especially doses earlier documented in preclinical mammalian testing.[34] In summary, the available pharmacokinetic data disapprove the use of ivermectin in the management of COVID-19, even though it is said to be a broad-spectrum antiviral agent, its potential is still *in vitro* and not *in vivo* as the SARS-CoV-2 inhibitory concentrations are practically not attainable in humans.[30]

The delivery of ivermectin by pulmonary route would provide high drug deposition in the airways and lungs. However, there is a need for investigations and new technology to overcome the mechanical, chemical, immunological and behavioural barriers that hinder the respiratory route of administration.

Efficacy and Safety of Ivermectin

Currently, the United States website ClinicalTrials.gov lists a total of 197 studies of Ivermectin worldwide, of which 81 related to SARS-CoV-2 or COVID-19 infection or sequelae. As of this writing, one trial to reduce viral load is underway, and 50 studies are not yet recruiting, recruiting by invitation only, or actively recruiting new study subjects.[i] Ivermectin has an established safety for human use and is FDA approved for some parasitic diseases.[17] The clinical efficacy of ivermectin in the treatment of COVID-19 has remained difficult to predict as we are dealing with a novel and frequently mutating virus.[20] Ivermectin has shown to be efficacious in the treatment of adult COVID-19 patients with mild symptoms.[16,35] The safety of ivermectin in animals and humans has been shown after oral, subcutaneous or topical administration at the recommended dose range (150-200 μg/kg in humans and 6-500 μg/kg in animals depending on species and formulation) and the indicated clinical applications. Within these dose ranges, pharmacokinetic characterizations have shown that attainable peak plasma concentrations increase with dose and may range from 20-81 ng/mL in humans.[31]

A study reviewing the safety and tolerability of escalating doses of ivermectin in healthy humans showed that a single dose (120 mg) that is 10-fold more than the clinically recommended dose (200 μg/kg) was well tolerated and it yielded a peak plasma concentration equivalent to 248 ng/ml with an elimination half-life of 19-hours.[23,31] Similarly, population-based pharmacokinetic modelling showed that ivermectin administered orally for 3-days at 600 μg/kg would yield maximal median plasma concentrations of 105-119 ng/ml (0.12-0.14 μM) and an elimination half-life of 3-5-hours.[23] These results suggest that even with extremely high doses of ivermectin, attainable peak plasma concentrations would remain markedly lower than the established IC50 concentrations for most viruses *in vitro*, though significantly higher than 0.5-1 ng/ml that is widely considered optimal for curative anthelminthic activity. The use of extremely high doses of ivermectin increases the prospect of adverse drug-drug interactions in patients requiring polypharmacy, as is often the case in treatment of viral infections and other infectious diseases.[31]

A recent randomised clinical trial assessed the efficacy of ivermectin at a dose of 300 μg/kg of body weight per day for 5-days *versus* the placebo. The main outcome of the study was complete resolution of symptoms within 21-days and the secondary outcome was the frequency of adverse events that were observed after using ivermectin in comparison to the placebo cohort. The results from this study show that additional multi-center research may be needed to demonstrate more robust evidence of a role for the use of ivermectin in the management of COVID-19 as there was no statistical significance in the resolution of symptoms between ivermectin and placebo. The guidance provided by the WHO says that ivermectin should only be used in a research setting such as clinical trials.[36] Therefore, all individuals using ivermectin should do so only for clinical trial purposes.

i. National Institutes of Health (NIH) U.S. National Library of Medicine. Web site. https://clinicaltrials.gov/ct2/results?cond=&term=ivermectin&cntry=&state=&city=&dist=. Retrieved October 3, 2021. Accessed October 3, 2021.

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Association of Ivermectin with Lower Mortality Rates in Hospitalised Patients

The use of ivermectin for the treatment of patients hospitalised with COVID-19 infections of mild-to-moderate severity has previously been shown to significantly lower mortality and morbidity rates associated with infection.[24] In an urgent search for useful treatments, this has led to the "off-label" use of this drug in the management of COVID-19 (i.e. unapproved by regulators such as the United States FDA for that particular clinical use). Studies have been conducted to demonstrate the relationship between the use of ivermectin and lower mortality rates in hospitalized patients.

A multihospital retrospective, unmatched cohort study by Rajter et al[27] conducted at a four-hospital consortium in South Florida reported that there was a reduction in the mortality rate of COVID-19 hospitalised patients who were treated using ivermectin. This observation was especially seen in patients who required higher inspired oxygen or support for ventilation. Analysis of results indicated that the mortality rate for those treated with ivermectin was 15% *versus* 25% in the usual care arm, with a significance level of p=0.03. There was a significant association of ivermectin with improved survival for patients who were admitted with COVID-19.[27] The positive association was also seen in the subject subgroups with severe pulmonary disease and other comorbidities. It also showed that the mortality for the subgroup of patients who had severe pulmonary involvement was lower in the ivermectin treatment group (38.8%) compared to the usual treatment group (80.7%) from the standard of care subject arm of the study, at a significance level of p=0.001. The length of hospital stay was not significantly different between the Ivermectin treatment group and the regular treatment group.[27] Because the cohorts were not matched and the study was not prospective, this study presents methodological limitations that may limit more than limited generalization to other settings or populations.

Ahmed et al[37] revealed that early intervention with ivermectin for the treatment of hospitalized mild to moderate COVID-19 infected patients may lower the amount of viral replication in the host. Early intervention in people with mild infection was associated with faster viral clearance, suggesting improved host immunity as well as the presumed lessening of viral transmission. Another study has also shown a reduction in mortality in ivermectin-treated patients with severe COVID-19 pulmonary involvement.[38] According to the pilot study conducted by Pott-Junior et al,[16] ivermectin used with standard of care (SOC) treatment was seen to reduce the risk of COVID-19 progression. The patients who were treated with standard of care treatment plus ivermectin at 200 mcg/kg or more had better clinical outcomes compared to those treated with SOC alone. Additionally, another clinical trial investigated ivermectin's potential to prevent hospitalisations in individuals with early COVID-19. The efficacy of ivermectin to prevent hospitalizations was evaluated as primary outcome. The results showed that ivermectin had no significant effect on preventing hospitalization of patients with COVID-19 and patients who received ivermectin required invasive mitral valve surgery (MVS) earlier in their treatment than those who were on placebo.[39] These findings are in agreement with a similar study where there was no improvement in symptom resolution among those that received ivermectin compared to the placebo.[29]

On the contrary to no significant group differences in the trial stated above, ivermectin was found in Rajter et al[27] work to reduce mortality rates in patients with COVID-19 especially those with severe pulmonary involvement. However, the authors recommended randomised clinical trials to prove these findings. Another study in Egypt found a reduction in hospitalisation among patients who received ivermectin in comparison to those who received the standard of care alone.[40] Noted reductions in hospital stay and mortality shows the potential benefits of using ivermectin in the treatment of COVID-19.

Despite studies showing the association of ivermectin with lower mortality rates in the management of COVID–19, other studies have shown no improvement or effect on time-to-resolution of the symptoms. In the double-blind, randomised trial conducted at a single site in Cali, Colombia, 200 patients were randomised to receive ivermectin, 300 µg/kg of body weight per day for 5-days whereas 200 patients received the placebo for 5-days.[29] The most important outcome in this study was that 82% of participants on ivermectin had their symptoms resolve by day 10 while 79% of participants on the placebo had their symptoms resolve by day 12.[29] A 5-day treatment course of ivermectin among adults with mild COVID-19 did not significantly improve the time to resolution of symptoms compared with placebo. The findings, thus, did not support the use of ivermectin for the treatment of mild COVID-19.[29] Therefore, studies have produced different findings on the use of ivermectin in the treatment of COVID-19. This means that there is a need for adequate evidence supporting or disputing the use of ivermectin to treat COVID-19. This is also supported by the WHO conclusion on the ability of ivermectin to reduce mortality rate which was found to be of very low certainty hence the suggestion that the drug only be used within clinical trials until more data is available.[36]

CONCLUSION

The use of ivermectin in the treatment of COVID-19 has been supported by two studies. However, large clinical trials are needed to provide sufficient methodological rigor and unequivocal evidence for the clinical use of ivermectin in the treatment of COVID-19 in humans. Multi-center clinical trials are warranted to support the use of this drug in humans confidently and also to ascertain its safety at different therapeutic doses in the COVID-19 patient population.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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