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Pharmacokinetics and pharmacology to drugs used for control of emerging cryptosporidiosis and toxoplasmosis in livestock and humans

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Abstract
Cryptosporidiosis and toxoplasmosis are the two main emerging diseases of parasitic protozoan origin having a very wide range of hosts, including almost all mammals, birds, fish and reptiles which spread mainly *via* contaminated water and food. Large numbers of studies have described the parasitological, seroprevalence, molecular and geographic distribution of these organisms throughout the world. The importance and generation of great public health interest of these diseases is increasing day by day. As the chances of human-animal interaction get augmented in the changing scenario the probability of these economically important zoonotic infections also goes up. As at present no vaccine with 100% efficacy is available against these diseases, hence preventive and chemotherapeutic measures mainly rely on the effective use of various chemotherapeutic agents. Currently different drug for chemoprophylaxis and chemotherapy have been tried which include Nitazoxanide, Halofuginone, Paromomycin, Azithromycin, Rifaximin etc. against cryptosporidiosis. On the other hand for the preclusion of toxoplasmosis main drugs used include are Bisphosphonates: farnysyl diphosphate inhibitors, Bumped kinase inhibitors, Dihydrofolate reductase inhibitors, Endoplasmic reticulum-associated degradation inhibitors, Fatty acid synthesis inhibitors, Fluoroquinolone derivatives, Histone acetyl-transferase/histone deacetylase inhibitors, Macrolide derivatives, Peptide-conjugated phosphorodiamidate morpholino oligomers, 4-(1H)-quinolones and Thiosemicarbazones with capricious success rates. The main aim of this article is to discuss the current significance of available chemotherapeutic agents in relation to their effectiveness in order to cure or decrease the potential of economic losses caused these protozoan infections. Additionally historical background, pharmacokinetics, mode of action, various *in-vitro* and *in-vivo* trails aspects have also been discussed.

Keywords: Chemotherapy; pharmacokinetics, cryptosporidiosis and toxoplasmosis

1. Introduction

In the present world, the cause of life-threatening diarrhoea in neo natal bovine calves and children is a small apicomplexan protozoan parasite belonging to the genus *Cryptosporidium* which is responsible for 800, 000 deaths for kids below the age of 5 years annually [1]. It is graded as 2nd imperative protozoan pathogen connected with long-term growth stumbling and cognitive insufficiency which lead to childhood morbidity and mortality worldwide along with pessimistic long-term impact on escalation, body mass addition, and corporeal and mental maturity, electrogenic chloride secretion, increased intestinal permeability [2]. It causes mainly the diarrhoea, so the nutrition and mineral absorption power of intestine also decreases, and association with other intestinal pathogens result into aggravation of the infection. Some researcher had documented a proof of *C. parvum* and associated pathogens responsible for severe outbreaks of bovine calf diarrhoea culminating in heavy mortality from Northern India, as 43 per cent of the affected calves died within a week after collection of the samples [3]. The organism is highly resistant and several anti-coccidials in claves and humans have been tried with limited success. The 1st annotations about the genus *Cryptosporidium* was made in 1907 by Ernest Edward Tyzzer (1875-1965). This parasites mainly infect gastrointestinal (microvillus coating of intestinal epithelial cellular layer leading to atrophy of the infected and nearby villus, hyperplasia of crypt, and uneven augment in WBC in the lamina propria region) or respiratory tract of mainly all animals species which may include mammals, reptiles, avian and fish [1]. Cryptosporidiosis has now entered the vanguard of public notice as this infection became deadly in immunosuppressed persons like in case of HIV/AIDS patients.
In homo-sapiens the illness by *Cryptosporidium* sp is mainly cause by 2 important species:

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Cryptosporidium hominis and *Cryptosporidium parvum*. For a developing country like India, important public health allegation*s* are also somehow related to *Cryptosporidium*. It also causes traveller's diarrhoea which is mainly linked with contaminated water, like in polluted water parks, or the community water supply [1]. Infection with *Cyptosporidium* is directly linked with the immune system of an individual. Patent with week immunity develop severe disease and they requires vigilant supervision with antimotility drugs, electrolytes (Na, K, HCO_3- and $C_6H_{12}O_6$) and other fluids drips, antiretroviral therapy, and frequently antiparasitic chemotherapeutic agents. Many chemical compounds (Nitazoxanide, Halofuginone, Paromomycin, Azithromycin, Rifaximin etc.) have shown anticryptosporidial properties [2]. Therapeutic management of C*ryptosporidium*-related diarrhoea in bovine calves with anti-coccidial, antibiotics and antidiarrhoeal agents with parenteral fluid therapy gave better recovery (71.4%) compared with untreated control [4]. Metronidazole and furazolidone combination was able to induce clinically and parasitological recovery in bovine calves in Punjab, India [5]. A clinical trial in C*ryptosporidium* positive bovine calves [6] found that the antibiotic azithromycin and the anticoccidial tylosin were both able to combat the cryptosporidial infection. For the better results the anticryptosporidial amalgams require to pass through at least 3 covering obstacles, i.e., the epithelial casing of host, parasite covering and the membrane of parasitophorous vacuole. Therefore porosity is a influential subject for a complex to depict enhanced anti-cryptosporidial activity along with its well capturing ability by *in-vitro* HCT-8 (human colon carcinoma cell lines) infection assays with drug action 12-24 h following sporozoite infection [1].

2. Anti-cryptosporidial drugs
2.1 Nitazoxanide (NTZ)
In the year 1980s NTZ (Nitrothiazolyl-salicylamide derivative) was made by mingling a thiazole ring (similar structurally to metronidazole's similar compound) with a benzamidine ring (anti-cestodal drug niclosamide's similar compound). Initially it was developed as a veterinary anthelmintic compound; following research explain a broad antibiotic continuum, counting helminthes, viruses and protozoan parasites [2]. NTZ is abroad range anti-parasitic and is potently used as a deworming mediator as well as in inhibitory examination of giardiasis and cryptosporidiosis infection. FDA has approved this drug for the patient above 1 year of age, in case of under nourished kids this drug had improved diarrhoea and death rate [1]. Regarding the efficacy range in diseased mammals, this drug had various values, in vigorous adults it goes upto 80% and in under nourished children it goes upto 56%, but in immune compromised patients, even high-dose long-lasting treatment is not at all effective [2]. NTZ and its two metabolites, tizoxanide (TZ) and tizoxanide-glucuronide (TZ glu) were revealed to restrain the expansion of *C. parvum* at dose rate of less than 10 mg/L [7].
In homo-sapiens the research related to pharmacokinetics of drug revealed that this compound is engrossed from the GI lower route, and about 1/3rd of the oral amount is expelled out via urine and 2/3rd expelled out in faeces [2]. In circulating system, this drug is swiftly hydrolyzed by various enzymes like plasma esterases into its desacetyl derivative, tizoxanide (desacetyl-nitazoxanide). In various in-vivo studies it was reported that this desacetyl derivative is the active metabolite and is expansively attached to protein in plasma (99%), and

its urinary abolition half-life is 7.3 hrs [8]. Tizoxanide is then glucuronide conjugated into the active metabolite, tizoxanide glucuronide. In case of *Trichomonas vaginalis*, NTZ act as a non-competitive inhibitor of the pyruvate: ferredoxin/flavodoxin oxidoreductases (PFORs) moreover in *Escherichia coli* infection it is feebly active against the pyruvate dehydrogenase [8]. In protozoan infection some other mode of action of this drug are blockage of protein disulphide isomerases (PDI_2 and PDI_4), changed appearance of genes which are concerned in response to stress such as heat-shock proteins, and interface with a novel *Giardia lamblia* nitroreductase, G_lNR_1 [9].

2.2 Halofuginone
Halofuginone (HF) is a small molecule derived from a natural product, febrifuginea naturally occurring alkaloid found in the root of hydrangea plants. It is mainly active on the free stages of the parasite (sporozoite, merozoite). In veterinary medicine field at present in cattle and poultry this drug had been approved to handle cryptosporidiosis and coccidiosis caused by *Eimeria tenella* respectively [8]. In protozoan parasites the main site of HF action is cytoplasmic prolyl-tRNA synthetase. The use of HF in homo-sapiens had been banned because apart from the reports of powerful anti-cryptosporidium activity, HF leads to the toxicity of liver with GIT side effects [1]. The active substance in HF prevents the growth of *Cryptosporidium parvum*. It also prevents it from forming oocysts [8].

2.3 Paromomycin
It is an oral non absorbable aminoglycoside which was discovered from *Streptomyces krestomuceticus* in the 1950s and came into medical use in 1960 for treatment of protozoan disease i.e. amebiasis moreover this drug had also been successfully used in adults and children for the cure of cryptosporidiosis [7]. Absorption of this drug in GIT is poor and any impediment or factors which weaken GI motility may increase the absorption of the drug from the GIT [1]. Moreover any structural injure, such as lesions or ulcerations, will tend to increase drug absorption [8]. After intramuscular (IM) injection, the absorption is rapid. Within one hour following IM injection it will reach peak plasma concentration [7]. Approximately 100% of the oral dose is abolished unchanged via faeces. Regarding mode of action this drug prevent the process of protein synthesis and unite specifically to the RNA oligonucleotide at the A site of bacterial 30s ribosome, thus causing misreading and premature termination of translation, thereby leading to inhibition of protein synthesis followed by cell death.

2.4 Azithromycin
Azithromycin was first discovered in 1980, it is a macrolide antibiotic and a derivative of erythromycin. Its lactone ring has additional nitrogen, where methylation process takes place. These changes in the structural enhance the acid constancy and tissue diffusion and broaden the range of action [7]. Regarding its mode of action, like other macrolides, this drug has bacteriostatic activity and by binding 50s ribosomal unit of microbe it restrains the making process of protein [10]. It blocks the protein formation at translocation step [Newly created peptide tRNA moves from the acceptor (A) to peptidyl donor site (P) on the ribosome] [11]. On the other hand, this compound can also join and cause a conformational transformation that cease the making of protein by two ways

i.e. indirectly nosy with transpeptidation and translocation process. Therefore the formation of proteins get restrained [12]. Regarding its pharmacokinetics, it is an acid-stable antibiotic, rapidly absorbed, after oral intake with 40% bio available [7]. After intravenous dosage its highest concentration in plasma occurs at 1-2 hrs. Amount of drug present in blood is lower than in tissues. Quantity of drugs in leucocytes, phagocytes and fibroblasts get supplied to the inflamed tissues region [12]. At very low plasma concentrations the drug binding ability to protein is 50% and it gets even less at higher concentrations moreover it can also crosses the placenta [7]. It goes through some liver metabolism process to inactive metabolites, but it suffers primarily biliary emission [10]. By urinary system about 6-12% of unaltered drug get excreted out of the body. Azithromycin has also reported to clear cryptosporidium infection in many cases. Moreover in some patients this drug has also been used to treat in immune-compromised hosts which are having reduced stool occurrence along with amalgamation with nitazoxanide and/or paromomycin [13].

2.5 Rifaximin
In year 1987, Italy was the first country in which Rifaximin (semi-synthetic compound of rifamycin) was permitted. In 2014 USA-FDA approved this drug by the name of Xifaxan (Salix Pharmaceuticals) for the action against cryptosporidiosis [14, 15], and is currently accepted for use in 17 nations [16]. This drug is not effected by gastric fluids, and is feebly engrossed, after oral administration with a bioavailability of <0.4% in circulating system [17]. Regarding its excretion as unaltered form from the body, about 97% come out in the faeces, 0.32% in urine and with no traces in bile or breast milk. For the control of *C. parvum* infection various in-vitro tests had been conducted using rifabutin, it shows the 25% case reduction alone but when used along with nitazoxanide, infectivity decreased by 75% [15].

Regarding mode of action, it inhibits transcription and RNA synthesis by attaching the beta-subunit of microbial RNA polymerase [18]. After oral administration it proceeds in the vicinity of GIT and has insignificant incorporation (plasma concentration is 0.01%) and get excreted via urinary system (less than 0.01%) in unchanged form [17]. As a result, there is negligible danger of toxicity or any systemic adverse effects. This drug is not soluble in H_2O and has more hydrophobic properties so throughout the GIT region its bioavailability always fluctuates [15]. In the existence of bile acids the solubility of the drug gets augmented by hundred fold, which in turn propose that its antimicrobial action is mainly accomplished in the small intestine than in the colon [16]. By measuring the diminished intensity of interleukins (IL-17 and IL-6) and tumour necrosis factor alpha (TNF-α) it was also concluded that this drug also decreases mucosal inflammation along with visceral ache in reply to chronic stress [15, 19]. The intestinal microbes also uses various mode to enhance their pathogenicity level like they get attached to epithelial cell lining and internalize, or overrun, the host healthy cells, all these process can also be obstructed by Rifaximin [19]. Cells mainly of epithelial layer when treated with rifaximin revealed decreased entero-aggregative *E. coli* attachment as well as diminished addition and trans-location of *Bacillus anthracis* or *Shigella sonnei* [19].

2.6 Antiretroviral treatment and other targets
When patients have access to active antiretroviral treatment, there is a reduction in the disease incidence [20]. From Europe and Australia, the data has been analysed from almost 7000 patients, and it was observed that the hazard for cryptosporidiosis diminished by 96% after the prologue of inhibitor of protease enzymes and combination remedy [11, 20]. It is observed that in the life cycle of the *Cryptosporidium* proteases play an important role and is involved in many crucial steps, so is the inhibitor of protease enzymes, such as α-1-antitrypsin, when these were added to in-vitro culture of cell, they will result in inhibitory effect on excystation and reduce in the amount of *Cryptosporidium*-spoiled cells [21]. Moreover another enzyme i.e. Clan CA cysteine proteases have also been established to be a possible aim, as per the notion they were imperative for host cell assault and has been found to be structurally dissimilar than equivalent enzymes in homo-sapiens [21, 22]. When this protease was inhibited utilizing N-methyl-piperazine-Phe-homo Phe-vinyl sulfone phenyl (K11777), it leads to reduction in infection [23]. Moreover by using HIV protease inhibitors saquinavir, ritonavir and indinavir in both *in vitro* and *in vivo* cultured test with *C. parvum,* it is seen that the infection depicted a dose-related outcome on the diminution of illness in cell cultures and in mouse [24, 25].

For the treatment of cancer folate biosynthesis pathway are been targeted, this pathway can also work in reducing cryptosporidial infection [11]. Cryptosporidium have an important enzyme i.e. bi-functional thymidylate synthase/dihydrofolate reductase. In in-vitro culture various scientists are assessing the action of amalgam intended to particularly inhibit and obstruct this main enzyme in the folate synthesis pathway [11]. Cryptosporidium use CDPK1 (calcium dependent protein kinases) as an indispensable element of cell invasion process [22]. This organism also lacks amino acids jamming access to the active site by "bumped kinase inhibitors". Researchers have recognized some complex that attach to this enzyme, restraining its purpose and which lead to the assassination of Cryptosporidium cell [23].

Another probable medicinal objective is acting on oxidoreductase inosine 5′-monophosphate dehydrogenase (IMPDH), it is necessary in the synthesis of guanine. Dissimilar to human IMPDH, CpIMPDH appear to evolve from bacteria via gene transportation technology, therefore its structure is an entirely different form human part. Contemporary study has established a chain of inhibitors for this enzyme [26, 27]. An additional investigation observed the use of Phylomer® peptides to hold back the purpose of Cp-IMPDH [11].

3. Toxoplasmosis
Toxoplasmosis is caused by *Toxoplasma gondii* a single-celled microscopic parasite fit in the phylum Apicomplexa that infects all warm-blooded vertebrates, including mammals and birds. This is the only single known species in the genus *Toxoplasma* and is considered as one of the most successful eukaryotic pathogens in terms of the number of host species and percentage of animals infected worldwide. The eponymous organelle of this phylum, the apical complex, is used to march into the cell of definitive host. Its definitive host is cat and intermediate host is all warm blooded creature in the planet. In various places throughout the world, it has been shown that approximate 95% of inhabitants have been fouled with this parasite. In immune competent hosts mainly more than 80% of primary *T. gondii* infections do not show any symptom [28]. For the first time this organism was exposed in 1908 and named after one year. Its human significance was

noticed in 1939, when it was recognized in tissues of a congenitally ruined newborn, and significance in animal field became recognized in 1957, when this parasite was found to be the main cause of abortion storms in sheep from New Zealand [29]. Subsequently there were reports of a similar disease in sheep occurring in other countries worldwide including Australia, UK and Europe. For the first time in India, Shastri, [30] isolated oocysts from the faeces of a stray cat which were later validated biologically by infecting Wistar rats [31].

In year 1948 the finding of a *T. gondii* precise antibody test in Sabin-Feldman dye test lead to the identification that *T. gondii* is a widespread coccidian having cosmopolitan allocation. Its complete life cycle was not exposed until 1970, in this year it was establish that felines are its ultimate host and an environmentally immune phase (oocyst) is emitted in faeces of diseased *Felis catus* [29]. General mode of transmission is by consumption of inadequately cooked groceries that have oocysts, contact to contaminated cat faeces, moreover if mother is infected during pregnancy then also child can become infected and sometime also by blood transfusion. Mainly in adults this disease generally causes no obvious symptoms. It occurs in three forms: acute, chronic and latent. Toxoplasmosis can also be congenital, and is related with foetal loss and abortion, moreover within children, it is also connected with neurologic deficits, neurocognitive shortfall, and chorioretinitis.

T. gondii causes abortion, still birth and neonatal mortality in small ruminants. Cattle and horses are more resistant to toxoplasmosis than other livestock species. Neuropathological changes (encephalomyelitis) in foetus and anorexia due to severe placental necrosis play an important role as the cause of death of the foetus. Goats suffer severely than sheep and affected adult goat can die of the disease. Disease in adult sheep is rare. Clinical symptoms in sheep include foetal death, production of a mummified foetus, still born lamb or birth of a live but weak lamb [32]. A significant factor in determining severity of disease is the stage of gestation when infection occurs, the earlier in gestation the more severe the consequences for the developing foetus.

Since this disease cause great threat to both animal and human so it's proper control is very much essential. It can be prevented by combination effort of drug, vaccine and management approach.

4. Anti-toxoplasmosis drugs
4.1 Bisphosphonates: Farnysyl diphosphate inhibitors
These are a group of compound that avert the loss of bone compactness and are used for the handling of osteoporosis and other sickness of bone resorption. They are structurally alike to pyrophosphate and impersonate pyrophosphate's assembly, thus hold back the activation of enzymes that use pyrophosphate [33]. There attentiveness comes from the two phosphonate set, which work jointly to organize Ca ions and preferentially "attach" and bind to it. In case of osteoclasts, the main site is the mevalonate pathway containing nitrogen-containing bisphosphonates, e.g. alendronate, which hinder farnesyl pyrophosphate synthase [33]. For the making of sterols and polyisoprenoids *T. gondii*, retain a mevalonate pathway. The main subdivision of this alleyway is the amalgamation of farnesyl diphosphate, a forerunner to ubiquinone, sterols, and prenylated proteins. In *T. gondii* making of farnesyl diphos-phate is catalysed by the bifunctional enzyme farnesyl diphosphate/geranyl geranyl-diphosphate synthase [34] and is

repressed by nitrogen-containing bisphosphonates. These bisphosphonate now a day's consist of 2 classes: The N-containing and non-N-containing bisphosphonates and both these kind of bisphosphonates work another way. Regarding the pharmacokinetics, the drug which is reabsorb (from oral preparation) or injected (for intravenous drugs), around fifty percent is expelled untouched by the excretory system. The residue has a very elevated attraction for bone tissue, and is swiftly taken up into the bone surface [33].

4.2 Bumped kinase inhibitors (BKIs)
They are a type of anti-*T. gondii* complexes that specifically mark the *T. gondii* Ca-dependent protein kinase 1 (TgCDPK1), which is a component of the serine/threonine protein kinase family. TgCDPK1 controls the ca-dependent trail of *T. gondii* microneme discharge and is necessary for sashaying movement, host-cell assault, and way out [35]. *T. gondii* growth can be inhibited by chemical compound hindrance of TgCDPK1 which in turn prevent host-cell attack [36]. Mitogen-activated protein kinase of *T. gondii* e.g. 1 (TgMAPKL1) has also been recommended as a subordinate objective for the BKI, 1NM-PP1, as a sudden transformation in *Tg*MAPKL1, was linked with reduced vulnerability to 1NM-PP1 and alike BKIs [37]. The aim of 1294 is TgCDPK1, as established by an 11-time resistance to 1294 produced by an amino acid replacement (G128M) at the "doorkeeper remains" of TgCDPK1. At this location TgCDPK1 also comprises a small glycine residue, but human kinases have big remains. The extra room attained by the remains of glycine in TgCDPK1 has been oppressed for the plan of strong and choosy ATP-competitive TgCDPK1 inhibitors [38]. Abundant analogs of BKI also been established in the region with diverse centre gallows that have promising both in-vitro and in-vivo results [39]. Strong TgCDPK1 inhibitors also were created from pyrazolopyrimidine (PP) and 5-aminopyrazole-4-carboxamide (AC) gallows. Inhibitors of Pyrazolopyrimidine with 6-alkoxy-2-naphthyl cluster at the 3rd carbon location and a 4-piperidinylmethylene set at the 1 nitrogen place are >15,000-fold more vigorous against ATP-binding pocket of TgCDPK1.

4.3 Dihydrofolate reductase (DHFR) inhibitors
Like numerous other protozonal parasites, *T. gondii* also has a distinctive bifunctional DHFR-thymidylate synthase (TS) that comprises both catalytic positions on the similar protein. It hinders the role of dihydrofolate reductase, and is a kind of antifolate [40], because folate is required by quickly multiplying cells to make amino acid like thymine, this can result in treatment of disease. One of the first-line mediators presently used for treating toxoplasmosis, DHFR is a broadly studied drug goal that is inhibited by pyrimethamine. Medical utilization of pyrimethamine is mainly restricted by the blockage of host folate metabolism, which can lead to the reduction of WBC. The new class of DHFR inhibitors i.e. dihydrotriazinesis are effective against *T. gondii* and at IC50 of 20 nM, the dihydrotriazine JPC-2067-B [40] cause reduction in in-vitro expansion of *T. gondii*. Despite of having reduced oral bio-availability this compound was effectual in a mouse form of toxoplasmosis.

4.4 Endoplasmic reticulum-associated degradation (ERAD) inhibitors
In a membrane when proteins are inserted or given out from the cell suffer folding and post-translational alteration in the

Endoplasmic reticulum (ER), but amino portion of proteins turn into irrevocably mis-folded, and these are then reprocessed by an ubiquitin and proteasome linked process called ERAD [41]. For the recognition of mis-folded proteins and to attack them via ERAD pathway, mostly all eukaryotic cells have their own extensive method. In *T. gondii* this ERAD system is limited as comparative to other eukaryotes [42] making these organism more receptive to intervention with this alleyway. Researcher has monitored many other compounds which were found to attack different proteins in the ERAD pathway and concluded that inhibitors of signal peptide peptidase (SPP) were the most effectual and non-hazardous mediators based on in-vitro IC50s against malaria, such as NITD731, restrain *T. gondii* in human U-2 osteosarcoma (OS) cell line.

4.5 Fatty acid synthesis (FAS) inhibitors

Apicoplasts is the main site in *T. gondii* where the FAS take place. In prokaryotic and vegetation the elongation of young fatty acids is guided by a single multi-enzyme i.e. FAS II [43], but in animals and fungi FAS I pathway is found and it has sole big multiuse polypeptide that guide the steps in fatty acid elongation [44]. In the whole process of FA elongation, the end step is controlled by FAS II enzyme enoyl-acetyl carrier protein reductase (ENR), and this has been targeted by many drugs in order to block fatty acid synthesis. ENR is repressed by the antibacterial compound like triclosan (after attaching it amplify the enzyme's likeness for nicotinamide adenine dinucleotide (NAD$^+$) therefore there is development of a stable, triple based complex of ENR-NAD+-triclosan, which is not capable to take part in FAS), which hinder the in-vitro expansion of *T. gondii* at low mM to nM concentrations [45]. In this pathway, there are many other enzymes which can be targeted like β-ketoacyl-acyl carrier protein synthase; it is repressed by thiolactomycin, which is a naturally occurring thiolactone [46]. Electron microscopy of treated organism with this drug exposed distended mitochondrial, engorged Golgi complex cisternae, and unfinished separation of multiplying daughter cells.

4.6 Fluoroquinolone derivatives

These compounds are derived from quinolones and have a F (fluorine) atom joined to the central ring structure, characteristically at the 6-position or 7th carbon position. There mode of entry inside cell is via porins and, so are frequently used to cure intra-cellular harmful organism [47]. These compounds act on DNA gyrase and DNA topoisomerase IV and inhibit them, therefore now days they are extensively used in human and veterinary medication as bactericidal agents (many gram-positive bacteria) [48]. *T. gondii* can be controlled by Trovafloxacin [47] but now due to its liver side effect (hepatic failure) this drug is no longer used. Exact mode of action of these compounds in case of *T. gondii* is still not known, but it is supposed to hinder DNA formation in the apicoplast region.

4.7 Histone Acetyltransferase/histone deacetylase inhibitors

In many organisms like *T. gondii* modification in transcription can be done by the epigenetic control of gene expression by using post-translational modification of histone proteins. This post-translation modification can be done by acetylation of preserved histone lysine residues by histone acetyltransferases (HATs) and it usually increase transcription of the target gene [49]. Contrariwise, post-translation alteration can be removed by the histone deacetylases (HDACs) which perform de-acetylation therefore reducing the transcription of the specific gene. As life cycle of *T. gondii* has multiple stages so it requires noteworthy alteration in gene appearance, and many researchers have recommended many new therapeutic agents that obstruct this epigenetic change there by eliminating the parasite [49]. In *T. gondii* the cyclic tetrapeptide FR235222 result in hyper-acetylation of histone H^4 region, and thus hold back the growth rate of the organism, and is related with the transformation from the tachyzoite to bradyzoite stage. Moreover, TgGCN5b is important for tachyzoite reproduction [50], and a freshly introduced HAT inhibitor like garcinol [a polyisoprenylated benzophenone derivative take out from the kokum fruit (*Garcinia indica*)] can targets that HAT TgGCN5b.

4.8 Macrolide derivatives

Most of organisms from phylum Apicomplexa acquire an exceptional form of organelle that consists of a kind of plastid called an apicoplast, and an apical complex structure. The organelle is an adjustment that the apicomplexan applies in infiltration of a host cell [51]. This apicoplast is the place of numerous important metabolic pathways; mostly contain probable chemotherapeutic goals [52]. The main mode of action of these derivatives is blockage of bacterial protein bio-synthesis. So any molecules that hold back protein process in the apicoplast region, can also act as effectual agents against *T. gondii*. Clindamycin blocks the microbial ribosome at 50s sub-unit site and can also be used treatment of toxoplasmosis [51]. Other compound which can targets of 50s sub-unit are erythromycin and azithromycin and are regularly recommended for the cure of prokaryotic disease. Azithromycin avert fatality caused due to acute toxoplasmosis and has a long half-life [52]. Moreover it also causes a delayed death phenotype [53] i.e. in first round of reproduction, this drug act on the organism humbly, but after first round it completely block the further replication in all daughter organism, even after the removal of azithromycin.

4.9 Peptide-conjugated phosphorodiamidate morpholino oligomers (PMOs)

They are artificial oligomers that interfere with gene expression by binding with complementary mRNA sequences [54]. PMOs can play a vital role in disrupting the translation of main parasitic proteins. By means of a similar approach some researcher [55] joined a variety of PMOs to arginine octomers, generating peptide-conjugated PMOs (PPMOs). Moreover, some scientist also established specific knockdown of *T. gondii* DHFR, enoyl-acyl carrier protein reductase, and the transcription factor AP2XI-3, a main controller of bradyzoite differentiation [54].

4.10 4-(1H)-quinolones

In several apicomplexan parasites, like *Toxoplasma gondii*, *Plasmodium falciparum* and *Babesia microti* the cytochrome $bc1$ complex ($bc1$) is a drug target site. This complex diminish cytochrome *c* as part of the electron transport chain and produce an electrochemical pitch by relocating protons to the inter-membrane region moreover it also generate ubiquinone for pyrimidine bio-synthesis [56]. The $bc1$ Qo site oxidizes ubiquinol and the $bc1$ Qi site reduces ubiquinone. Qo-site inhibitor i.e. Atovaquone is now a day's used as a substitute treatment for toxoplasmosis [56]. Now a day's Qi site inhibitors are not in medical due to their cardiotoxicity in

mice and activity against human *bc*1 [56]. The endochin-like-quinolone (ELQ) series of 4(1H)-quinolone-3-diarylethers are originated from endochin, their goal is Qi site and have been planned to pass up human *bc*1 inhibition. In 1948 endochin was originally examined as an anti-plasmodial compound in birds [57]. ELQ-316 and ELQ-271 were found to be extremely effective for treating acute and latent toxoplasmosis. The compound 1-hydroxy-2-dodecyl-4(1H) quinolone (HDQ), a structural analog of ubiquinone, has been found as an inhibitor of type II NADH dehydrogenase was obtained from the yeast *Yarrow lipolitica*, *Saccharomyces cerevisiae bc*1 Qi site and *T. gondii* dihydroorotate dehydrogenase [58, 59]. The ETC of *T. gondii* contains a single subunit type II NADH dehydrogenase in place of the multi-subunit type I NADH dehydrogenase found in animals.

4.11 Thiosemicarbazones
In the management of polycythemia vera (slow-developing cancer of blood in which bone marrow create too many RBC) as well as other myelo-proliferative disorders an antineoplastic drug i.e. Hydroxyurea, or hydroxycarbamide is mainly used. In animal cells, its mode of action is by restraining ribonucleotide reductase [60], this enzyme perform the rate-limiting step in DNA formation. Hang-up of this enzyme, cause the stoppage of cell cycle at the G_1/S stage. Some researcher established that if we keep infected vero cells for 5hrs in 4 mM hydroxyurea then this drug can obstruct with intracellular organism duplication process [61]. This compound cause brutal morphologic modification to intracellular organism followed by the removal of organism from parasitophorous vacuoles.

5. Conclusions
Due to inadequate accessibility of the drugs against these zoonotic and veterinary pathogens, there is need for sensible use of drugs in combination with is feasible managemental practice so as to decrease the chances of drug resistance because once the drug resistant had developed then the present drug will become waist moreover it take many decades to finish a new drug trail and there is huge economic loss also.

6. Conflict of interests
All authors are having no conflict of interest.

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