Endochin-like quinolone-300 and ELQ-316 inhibit Babesia bovis, B. bigemina, B. caballi and Theileria equi

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Abstract

Background: The most common apicomplexan parasites causing bovine babesiosis are *Babesia bovis* and *B. bigemina*, while *B. caballi* and *Theileria equi* are responsible for equine piroplasmosis. Treatment and control of these diseases are usually achieved using potentially toxic chemotherapeutics, such as imidocarb diproprionate, but drug-resistant parasites are emerging, and alternative effective and safer drugs are needed. Endochin-like quinolones (ELQ)-300 and ELQ-316 proved safe and efficacious against related apicomplexans, such as *Plasmodium* spp., and ELQ-316 was also effective against *B. microti,* without showing toxicity in mammals.

Methods: Inhibitory effects of ELQ-300 and ELQ-316 were assessed on the growth of cultured *B. bovis*, *B. bigemina*, *B. caballi* and *T. equi*. Percentage of parasitized erythrocytes was measured by flow cytometry. Effect of the ELQ drugs on the viability of horse and bovine peripheral blood mononuclear cells (PBMC) was assessed by monitoring cell metabolic activity using a colorimetric assay.

Results: We calculated IC_{50} ranging from 0.04 to 0.37 nM for ELQ-300, and from 0.002 to 0.1 nM for ELQ-316 at 72 h among all cultured parasites tested. None of the parasites tested were able to replicate in cultures in the presence of the ELQ-300 and ELQ-316 at IC_{100}, which range from 1.3 to 5.7 nM for ELQ-300 and from 1.0 to 6.0 nM for ELQ-316 at 72 h. Neither ELQ-300 nor ELQ-316 altered the viability of equine and bovine PBMC at their IC_{100} in *in vitro* testing.

Conclusions: ELQ-300 and ELQ-316 showed significant inhibitory activity on the main parasites responsible for bovine babesiosis and equine piroplasmosis at doses that are tolerable to host cells. These ELQ drugs may be viable candidates for developing alternative protocols for the treatment of bovine babesiosis and equine piroplasmosis.

Background

Tick-borne diseases caused by apicomplexan hemoparasites, such as *Babesia* and *Theileria*, impose serious economic impact on the cattle and horse industries worldwide [1, 2]. Babesiosis and theileriosis share similar acute disease signs, including anemia, loss of weight, anorexia and fever [3]. Usually, *Babesia* and *Theileria* are not eliminated in surviving animals and cause lifelong persistent infections. Important shared features among *Babesia* and *Theileria* species include a sexual reproductive cycle in their *Ixodes* arthropod hosts and asexual reproduction in the red blood cells (RBC) of their vertebrate hosts, a process that results in severe, potentially fatal hemolytic anemia [4-6].

Theileria equi and *Babesia caballi* are the etiological agents of equine piroplasmosis (EP), a disease that affects horses, mules, donkeys, and zebras worldwide [7]. EP imposes severe and costly restrictions in transportation of high-performance horses between endemic and non-endemic areas to participate in equestrian sporting events [3, 6]. No vaccines are currently available against *T. equi* and *B. caballi*, and considerable resources have been spent to develop drugs to treat animals against the harmful effects of acute EP and to prevent the loss of performance in chronically infected, high-value horses. Despite these

efforts, horses that survive acute infection, especially when caused by *T. equi*, become persistently infected, asymptomatic carriers, a condition that can be associated with the resurgence of outbreaks of EP worldwide [8].

B. bovis and *B. bigemina* are two main causative agents of bovine babesiosis (BB), an acute and persistent economically important disease of cattle that typically cause high mortality [1]. While *B. bigemina* is usually associated with relatively milder acute hemolytic disease, *B. bovis* is implicated in a more severe presentation of the acute phase of the disease, characterized by cytoadhesion of parasite-infected RBC in the brain capillaries, which resembles cerebral malaria and often leads to death [1].

Prevention and control of EP and BB have been typically achieved by controlling tick vector populations, the use of live attenuated vaccines in the case of BB, and chemotherapy. The live attenuated vaccines available to prevent acute BB, which are in use only in a limited number of countries, are only recommended for less than 1-year old animals and present several additional constraints, including the risk of reversion to virulence. Furthermore, cattle vaccinated with live attenuated vaccines may also become persistently infected with the parasites, and can serve as a reservoir for tick acquisition and transmission [9]. In addition, live vaccines can cause severe disease to immunocompromised and older cattle, which may be more susceptible to the attenuated vaccine strains [9]. Given these scenarios, some animals vaccinated with live *Babesia* vaccines also need to be treated with anti-babesial drugs to prevent the development of acute disease caused by virulent escapes within the population of parasites in the attenuated vaccine strains. Currently, babesicidal drugs are the only option available for preventing loses due to babesiosis in adult vaccine-susceptible animals that need to be transported from non-endemic to *Babesia* endemic areas. Altogether, these aspects highlight the importance of having reliable babesicidal drugs to control the spread of outbreaks and prevent development of acute disease in herds vaccinated with live attenuated *Babesia* vaccines.

Chemotherapy treatments based on diminazene aceturate and imidocarb dipropionate are the most effective and first-choice methods to manage animals with acute BB and EP [10, 11]. However, the efficacy of these drugs is highly variable and treated animals need to be monitored closely for adverse effects, especially when high doses are used for attempting clearance of the parasites, which is a usual occurrence for valuable horses affected by EP [12]. In addition to toxic side effects, and although specific resistance to imidocarb by *Babesia* and *Theileria* parasites was not yet documented, the potential for the development of drug-resistance by *Babesia* parasites to other drugs such as amicarbalide isethionate has been previously recorded [13]. Consequently, there is the need to search for new effective and less toxic alternative chemotherapeutics against BB and EP.

Endochin-like quinolone (ELQ) are potent selective inhibitors of the mitochondrial cytochrome bc_1 complex, as demonstrated in *Plasmodium falciparum*, the causative agent of the most severe form of human malaria [14-17]. ELQ compounds have shown to be highly effective against different species and multiple stages of *Plasmodium* [18, 19]. Importantly, ELQ-300 and ELQ-316 have been selected as pre-clinical anti-malaria candidates, considering their reasonable oral bioavailability at efficacious doses,

long half-life, and metabolic stability [18, 19]. A recent study also demonstrated the efficacy of ELQ prodrugs combined with atovaquone to treat experimental babesiosis caused by *B. microti* in the immunodeficient mouse model [20]. Data from this study showed that the combined therapy of ELQ and atovaquone resulted in complete clearance of the parasite with no disease recrudescence even more than 100 days after discontinuation of the treatment [20]. Based on these conclusive parasite inhibitory results, we evaluated the effect of ELQ-300 and ELQ-316 on the *in vitro* growth of *B. bovis*, *B. bigemina*, *B. caballi*, and *T. equi*. Strong inhibition of the development of all these parasites, coupled with the lack of toxic effects on host cells, suggests that these two compounds are promising candidates for future development of novel alternative therapies to control BB and EP.

Methods

Synthesis of ELQ-300 and ELQ-316

ELQ-300 and ELQ-316 were synthesized as previously described [19, 21] (Fig. S1). Both compounds were kindly provided by the Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, OR, USA. Purity of both ELQ derivatives was assessed at >99% by proton NMR and gas chromatography – mass spectrometry. ELQ-300 and ELQ-316 were diluted in 100% dimethyl sulfoxide (DMSO) to prepare stock solutions. Stock solutions were kept at RT until use. Working solutions were freshly prepared in parasite culture medium before adding to the parasite cultures.

Cultures of *B. bovis*, *B. bigemina*, *B. caballi* and *T. equi*

B. caballi Puerto Rico strain [18], *B. bovis* Texas strain [19], *B. bigemina* Puerto Rico strain [20] and *Theileria equi* Florida strain [21] were grown in long-term microaerophilous stationary-phase cultures and incubated at 37°C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2, as previously described [22-25]. *B. bovis* and *B. bigemina* were grown in 96-well plate using 180 µl per well of complete HL-1 culture media (pH 7.2; 2.38 g/L HEPES, 5 mL/L L-glutamine, 60 U/ml of penicillin G, 60 µg/ml of streptomycin, and 0.15 µg/ml of amphotericin B (Sigma) supplemented with 40% bovine serum. Cultures contained a suspension of 10% and 5% packed cell volume of bovine erythrocytes for *B. bovis* and *B. bigemina,* respectively. *B. caballi* and *T. equi* were cultured under similar conditions, but culture media were supplemented with 10% or 20% horse serum, respectively. In addition, *B. caballi* and *T. equi* cultures contained a suspension of 10% packed cell volume equine erythrocytes.

Parasite growth inhibition assay

Growth inhibition assays using ELQ-300 or ELQ-316 were performed on cultured *B. bovis*, *B. bigemina*, *B. caballi* and *T. equi* with a starting percentage of parasitized erythrocytes (PPE) of 0.2. Parasites were

grown as described above, using culture media containing different concentrations of ELQ-300 or ELQ-316 from 0.05 to 50 nM diluted in DMSO. Parasite cultures in the presence of DMSO (0.5 µl) and in the absence of the ELQ compounds were used as a positive control for parasite growth. Extra wells containing uninfected bovine or equine RBC were prepared and used as negative controls for the flow cytometric analysis. Fresh culture medium (150 µl/well) containing respective drug concentration was replaced daily to parasite cultures. These experiments were carried out in triplicate for each tested concentration and controls, over a period of 72 h. PPE was monitored daily by flow cytometry, as previously described [22, 23]. Fifty percent inhibitory concentration (IC_{50}) values were calculated for ELQ-300 and ELQ-316 at 24, 48, and 72 h by extrapolation in which there is a 50% reduction of the PPE in the wells containing the ELQs compared with the positive control wells using nonlinear regression (GraphPad Prism version 8.0.2 for Windows, San Diego, California, USA). Similarly, 100% inhibitory concentration (IC_{100}) values were also calculated at 72 h.

Flow cytometric analysis for detection of parasite growth in cultures

PPE of parasite cultures was determined by flow cytometry, as previously described [26, 27]. Briefly, 5 µl of cultures were collected from the bottom of the wells and centrifuged at 450 *xg* for 1 min at 4°C. Supernatant was discarded, and cell pellet was washed twice with 150 µl of phosphate buffer saline (PBS) pH 7.2. Then, cell pellet was suspended in 200 µl of 25 µg/µl hydroethidine (HE) (Invitrogen), incubated in 5% CO_2 incubator at 37 °C for 20 min in the dark, and washed twice with 200 µl of PBS to remove the excess of HE. After that, the supernatant was discarded, and the cell pellet was suspended in 200 µl of fresh PBS. Then, suspended cells were analyzed by flow cytometry using a Guava® easyCyte flow cytometer (Luminex) at a ratio of 800-1,000 cells/µl with 20,000 events collected. Results were analyzed by FCS Express v6 (De Novo Software). Normal, uninfected horse and cattle RBC were used as a negative control for the flow cytometric analysis.

Effect of ELQ-300 and ELQ-316 IC_{100} on parasite growth

In vitro growth inhibition assays were performed over a period of 8 days using starting PPE at 0.2% and 2%. Parasites were grown in the presence of calculated ELQ-300 or ELQ-316 IC_{100} values. Cultures growing in medium in the absence of ELQs and non-infected RBC maintained in medium only were used as positive and negative controls, respectively. Culture medium containing respective compound concentrations was replaced daily, 150 µl medium per well, for a period of 72 h. Then, parasites were cultivated in media only and split every 48 h for a period of 8 days. PPE was evaluated at 24, 48, and 72 h, and 8 days of culture by flow cytometry.

Cytotoxicity assay

Cytotoxicity of ELQ-300 and ELQ-316 in *ex vivo* peripheral blood mononuclear cells (PBMC) of bovine and equine were examined by exposing cells to the compounds at their calculated IC_{50} and IC_{100}. For the bovine PBMCs experiment, ELQ-300 IC_{50} and IC_{100} were 0.56 nM and 4.3 nM, respectively. And for the ELQ-316 IC_{50} and IC_{100} were 0.07 nM and 3.92 nM, respectively. For the equine PBMCs experiment, ELQ-300 IC_{50} and IC_{100} were 0.23 nM and 5.94 nM, respectively. And for the ELQ-316 IC_{50} and IC_{100} were 0.11 nM and 6.18 nM, respectively. Viability of bovine and horse PBMC was evaluated by monitoring cell metabolic activity using a colorimetric assay. Briefly, peripheral blood was collected from healthy cattle and horses via jugular venipuncture into Vacutainer® tubes containing ACD (Becton Dickinson) and PBMC were isolated using Histopaque® (Sigma) per standard protocol. Cells were then plated at $2x10^4$ cells/well in 96-well plates in complete Dulbecco's modified essential medium cDMEM (10% fetal bovine serum, 24 mM of HEPES, 2 mM of L-glutamine, 100 IU/ml penicillin, and 100 ug/ml streptomycin) and incubated with the ELQ compounds. The Cell Proliferation WST-1 reagent (Roche) was added to the cell cultures following the manufacture's protocol at 24, 48, and 72 h after exposure to the ELQ compounds. Absorbance at 440 nm was measured using an ELISA plate reader at 4 h after adding WST-1 to the cells. Cells in cDMEM in the absence of the ELQ compounds and cells exposed to DMSO only (1/400 dilution, which corresponds to the highest volume used on the diluted ELQs) were used as negative controls. PBMC exposed to concanavalin (Con) A diluted in cDMEM (5 µg/ml) (Sigma) and Draxxin® [22] were used as a positive control.

Statistical analysis

Growth of parasites in culture was analyzed using one-way ANOVA (GraphPad Prism version 8.0.2 for Windows, San Diego, California, USA). Values of $P<0.05$ were considered statistically significant concerning the effect of the ELQs on the parasite growth. Significant differences in PBMC viability were measured by Student's t test, and P values <0.05 were considered significant.

Results And Discussion

ELQ-300 and ELQ-316 inhibit the growth of *B. bovis, B. bigemina, B. caballi,* and *T. equi*

The effect of ELQ-300 and ELQ-316 on parasite growth, with a starting PPE of 0.2%, was evaluated using seven different concentrations of each compound, ranging from 0.05 to 50 nM. Both tested drugs significantly inhibited ($P<0.05$) the growth of *B. bovis*, *B. bigemina, B. caballi,* and *T. equi* (Fig. 1A-D and Fig. 2A-D). In addition, the inhibitory effect of ELQ-300 and ELQ-316 was found to be dose-dependent for all four parasites tested. Calculated IC_{50} and IC_{100} values of ELQ-300 and ELQ-316 for each parasite are shown in Table 1. Overall, comparisons of the IC_{50} values among all parasites tested indicate increased

susceptibility to ELQ-316 than to ELQ-300. The ELQ-316 IC_{50} varied from 0.002 to 0.1 nM, while in the ELQ-300 compound it varied from 0.04 to 0.37 nM, as measured at 72 h of culture (Table 1).

Interestingly, our calculated values of IC_{50} for ELQ-300 and ELQ-316 are in the same range or lower than values estimated for other related apicomplexans in previous studies. ELQ-316 IC_{50} values of 7.97, 0.66 and 0.35 nM were established for *Besnoitia besnoiti* and *Toxoplasma gondii* tachyzoites, respectively [28, 29]. In addition, a previous study demonstrated ELQ-300 IC_{50} values of 15.4 and 23.1 nM for *P. knowlsei* and *P. falciparum*, respectively [15]. Besides the acceptable IC_{50} inhibitory values found for ELQ-300, our study showed even lower ELQ-316 IC_{50} values for *B. bovis*, *B. bigemina*, *B. caballi*, and *T. equi*, suggesting that these parasites are also highly susceptible to these two drugs. In addition, the IC_{50} values obtained for ELQ-300 and ELQ-316 are lower than the values shown with anti-babesial drugs in recently published studies, but in the same IC_{50} range of imidocarb dipropionate for the *B. bovis* and *B. bigemina* (Table S1).

Consistently, ELQ-300 and ELQ-316 completely abrogated the growth of all four parasites when tested at their respective IC_{100}. The calculated IC_{100} values ranged from 1.3 to 5.7 nM for ELQ-300, and from 1.0 to 6.0 nM for ELQ-316 (Table 1). Overall, *B. bigemina*, displayed the lowest IC_{100} value out of the four parasites tested, and appears to be the most susceptible parasite to ELQ-300. On the other hand and based on the IC_{100} values (Table 1), *T. equi* appears to be more susceptible to ELQ-316 than the other four parasites tested in this study. Taking the IC_{50} and IC_{100} data together, ELQ-300 and ELQ-316 are able to efficiently inhibit the *in vitro* growth of *B. bovis*, *B. bigemina*, *B. caballi*, and *T. equi* blood stages. Notably, while the calculated IC_{100} of *T. equi* is unexpectedly high (500 times higher than the IC_{50}) (Table 1), we cannot rule out, however, the possibility that the actual concentration of the drug in the culture well was affected by poor solubility in the culture media.

Growth inhibitory effect of ELQ-300 and ELQ-316 is independent of initial parasitemia

We then tested whether the efficiency of the compounds is dependent on the parasite initial parasitemia, by comparing the effects of ELQ-300 and ELQ-316, at their respective IC_{100}, on the four parasites growing in *in vitro* cultures with starting PPEs of 0.2% and 2%. Neither *B. bovis, B. caballi* nor *T. equi* were able to grow in *in vitro* cultures in the presence of the IC_{100} ELQ-300, regardless of their initial PPE (P <0.05) (Fig. 3A, C, and D). Nonetheless, the addition of ELQ-300 to *B. bigemina* cultures with an initial PPE of 2% did not result in a rapid decrease of parasitemia (Fig. 3B), in contrast to what was found when the initial PPE was 0.2% (Fig. 3B).

Based on these results, a parasite rescue experiment was performed where the parasites were grown in culture in the presence of ELQ-300 for 3 days, then cultures were split 1:10, and maintained in media free of the drug for 5 additional days. Parasite growth was not detected (P <0.05) by the end of this period of time for *B. bovis* and *T. equi*, but that was not the case for *B. bigemina* and *B. caballi* (Fig. 3B and C). These results suggest the absence of pre-existing ELQ-300-resistant parasite subpopulations in the *B.*

bovis and *T. equi* strains with the ability to survive the initial drug-inhibitory treatment among the parasite strains tested. Collectively, these results are consistent with the relatively increased tolerance of *B. bigemina* and *B. caballi* to ELQ-300, compared to the other two parasites tested, as shown in Fig. 1B and C.

Interestingly, none of the four species of parasites tested in this study was able to grow in the presence of the ELQ-316 IC_{100} concentration regardless of their initial PPE at 72 h (P <0.05) (Fig. 4A-D). The same lack of parasite growth was observed after 8 days in the parasite rescue experiment, except for *B. caballi* (Fig. 4A-D), independent of the starting PPE. A possible interpretation of these results is that the *B. caballi* strain used in this study may contain a mix of subpopulations of parasites, each one with distinct degrees of tolerance for ELQ-316. In contrast, the *B. bovis*, *B. bigemina*, and *T. equi* strains used in these experiments appear to be composed of subpopulations that are highly susceptible to ELQ-316. It was beyond the scope of this study to investigate the mechanism involved in the susceptibility for the ELQ drugs. However, one may speculate that such susceptibility can be due to variations/mutations in the cytochrome bc1 target sequence that affect the ELQ binding, differential uptake or elimination of the drugs, or a combination of these factors [29-31]. It was recently shown that genetic alterations in the Q_i binding site of cytochrome *bc1* complex (*Cytb*) of *B. microti* is associated with resistance to ELQ-316, which suggests that this cytochrome gene is as a potential target for the ELQ drugs [16]. Based on these observations, we performed alignment analysis of the *Cytb* genes of *B. bovis*, *B. bigemina*, *B. caballi*, and *T. equi* together with the *B. microti Cytb*. Our results indicated full conservation of the two canonical Q_o and Q_i binding sites of *Cytb* in all sequences analyzed and a high level of amino acid identity, which ranged from 47.2 to 49.6 % in comparison to *B. microti* (Fig. 2) (Table S2). Overall, the results presented here set the rationale for further studies to alter and/or knock down the *Cytb* gene in these parasites and evaluate its potential effect on the susceptibility or resistance to the ELQ drugs.

ELQ-300 and ELQ-316 do not affect viability of equine and bovine PBMC

Cytotoxic assays were performed to assess whether ELQ-300 and ELQ-316 affect the viability of equine and bovine PBMC, which we used as surrogates of nucleated vertebrate host cells. The cytotoxic assays were performed using the IC_{100} doses of ELQ-300 and ELQ-316 in *in vitro* cultures. For the bovine PBMC experiment, ELQ-300 IC_{100} of 4.3 nM and ELQ-316 of 3.92 nM were used, respectively, whereas for the equine PBMC experiment, ELQ-300 IC_{100} of 5.94 nM and ELQ-316 IC_{100} of 6.18 nM were used, respectively. Viability of PBMC was similar regardless of the presence or absence of parasite lethal doses of ELQ-300 or ELQ-316, strongly suggesting that cell viability was not compromised by any of these two drugs under the experimental conditions used in the assays (Fig. 5A and B). In addition, significant increase (P <0.05) in cell proliferation was observed in bovine and horse PBMC exposed to ConA for 24 h and 48 h, respectively (Fig. 5A and B), indicating adequate sensitivity for the WST-1 proliferation assay used in this study. Taken together, results of cell viability revealed that ELQ-300 and ELQ-316, at their respective IC_{100}, lack significant toxic effect on *in vitro* cultivated bovine and horse PBMC.

Conclusions

Overall, results presented here demonstrate that both drugs tested in this study, ELQ-300 and ELQ-316, are efficient in inhibiting the growth of *in vitro* cultured *B. bovis*, *B. bigemina*, *B. caballi* and *T. equi*. Importantly, IC_{100} doses of the ELQ drugs did not significantly affect the viability of *in vitro* cultured cattle and horse PBMC. Collectively, findings of this study strongly suggest that ELQ-300 and ELQ-316 can be potentially effective and safe candidates for the development of novel therapies to control BB and EP. Further studies *in vivo* using horses and bovines are needed to evaluate the efficacy of ELQ-300 and ELQ-316 against acute and chronic BB and EP.

Abbreviations

Con A: concanavalin A

DMSO: dimethyl sulfoxide

ELQ: endochin-like quinolones

HE: Hydroethidine

IC: inhibitory concentration

nRBC: non-infected erythrocytes

PBMC: peripheral blood mononuclear cells

PBS: phosphate buffer saline

PPE: percentage of parasitized erythrocytes

Declarations

Availability of data and material

The datasets supporting the conclusions of this article are included within the article and its additional files.

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Author's contribution

MGS, RGB and CES conceived and designed the study. MGS and RGB performed the inhibitory assays and microscopy experiments, analyzed the data and drafted the manuscript. MGS and RGB performed the flow cytometry assay and cytotoxic assay. JSD, MKR, SP, RW, and AN manufactured and provided endochin-like quinolones, and gave technical guidance regarding their use. MGS, RGB, CES, JSD, and MKR wrote the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1 IC_{50} and IC_{100} of ELQ-300 and ELQ-316 calculated for *B. bovis*, *B. bigemina*, *B. caballi* and *T. equi* at 72 h of culture. Results are presented as mean and standard deviation based on triplicates for each experiment.

	ELQ 300		ELQ 316	
Species	**IC_{50} (nM)**	**IC_{100} (nM)**	**IC_{50} (nM)**	**IC_{100} (nM)**
B. bovis	0.09 ± 0.002	4.2 ± 0.10	0.07	3.8 ± 0.12
B. bigemina	0.37 ± 0.19	1.3 ± 0.05	0.05	3.0 ± 0.15
B. caballi	0.19 ± 0.04	5.7 ± 0.24	0.1 ± 0.006	6.0 ± 0.18
T. equi	0.04 ± 0.003	3.36 ± 0.83	0.002	1.0 ± 1.55

Figures

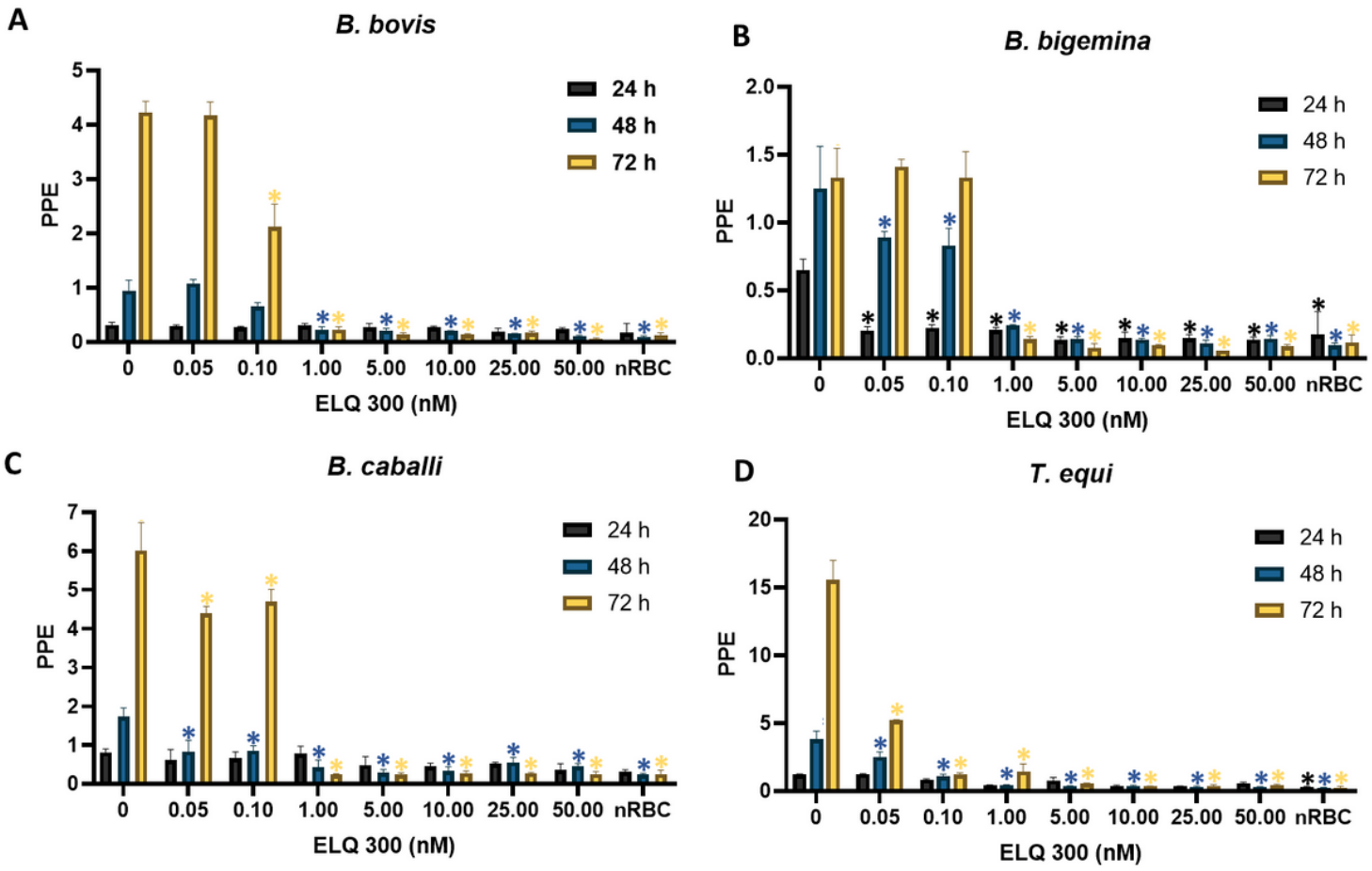

Figure 1

Parasite culture growth at 24 h (purple bars), 48 h (blue bars), and 72 h (yellow bars) without and after addition of different concentrations of ELQ-300. (A) B. bovis (B) B. bigemina (C) B. caballi. (D) T. equi. “0” represents parasites in the absence of ELQ-300. Assays were carried out in triplicate and the error bars indicate standard error deviation for each ELQ-300 concentration tested. (*) Represents P-value <0.05, indicating statistically significant difference between cultures grown without and with ELQ-300, using Student’s t-test.

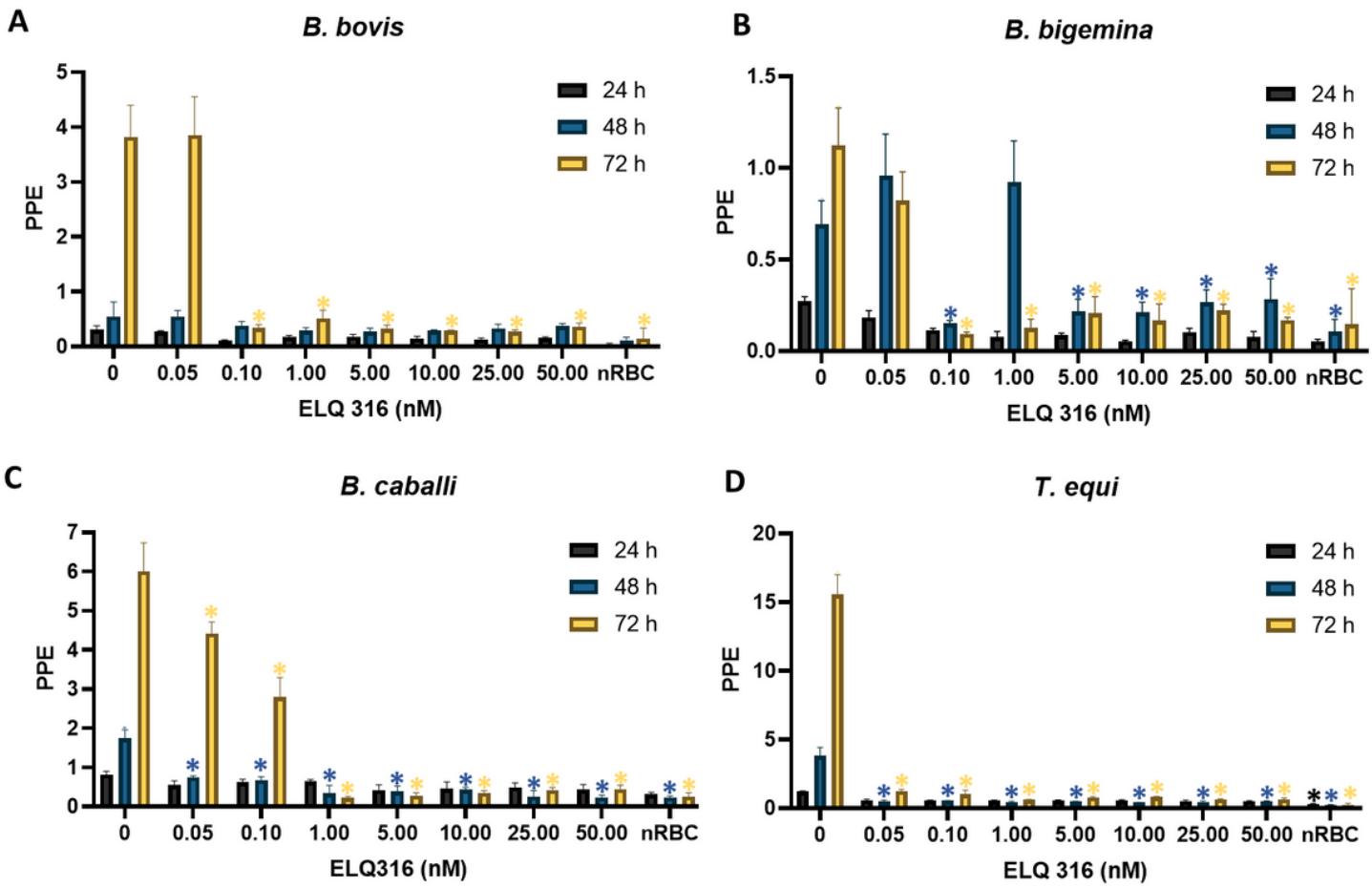

Figure 2

Parasite culture growth at 24 h (purple bars), 48 h (blue bars), and 72 h (yellow bars) without and after addition of different concentrations of ELQ-316. (A) B. bovis. (B) B. bigemina. (C) B. caballi. (D) T. equi. “0” represents parasites in the absence of ELQ-316. Assays were carried out in triplicate and the error bars indicate standard error deviation for each ELQ-316 concentration tested. (*) Represents P-value <0.05, indicating statistically significant difference between cultures grown without and with ELQ-316, using Student’s t-test.

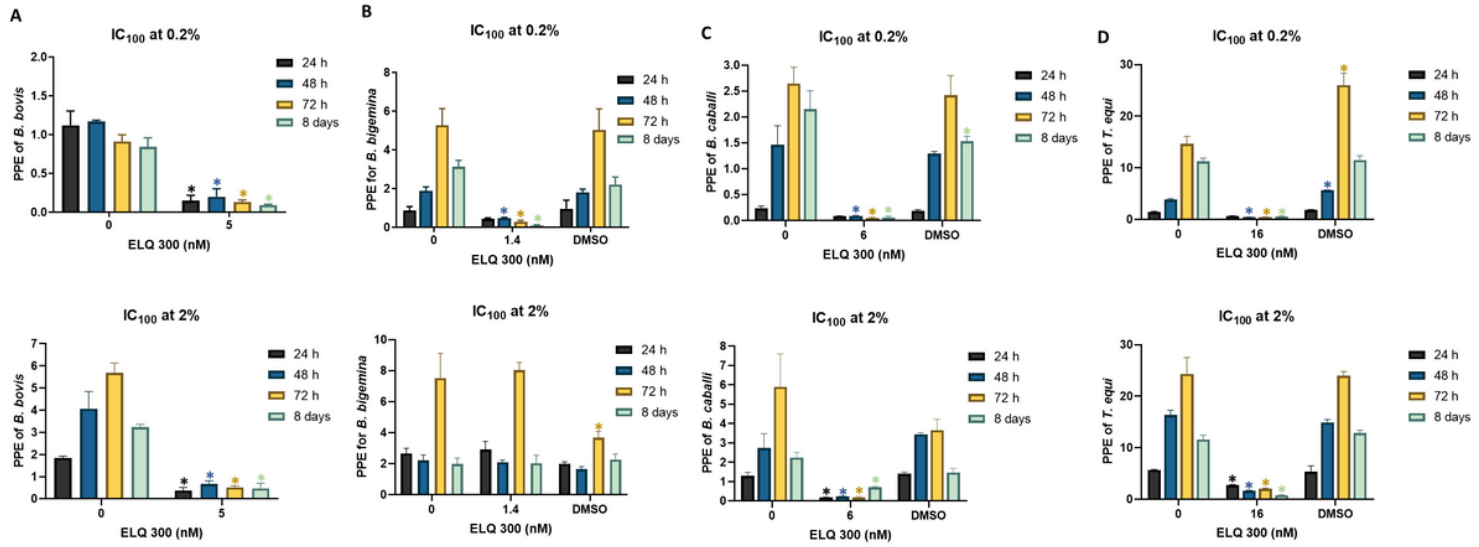

Figure 3

Parasite culture growth at 24 h (purple bars), 48 h (blue bars), and 72 h (yellow bars) using IC100 of ELQ-300, and 8 days (green bars). (A) B. bovis. (B) B. bigemina. (C) B. caballi. (D) T. equi. Upper panel represents 0.2% PPE, lower panel 2% PPE. “0” represents parasites grown without addition of ELQ-300. Assays were carried out in triplicate and the error bars indicate standard error deviation. (*) Represents P-value <0.05, indicating statistically significant difference between cultures grown without and with ELQ-300 using Student’s t-test.

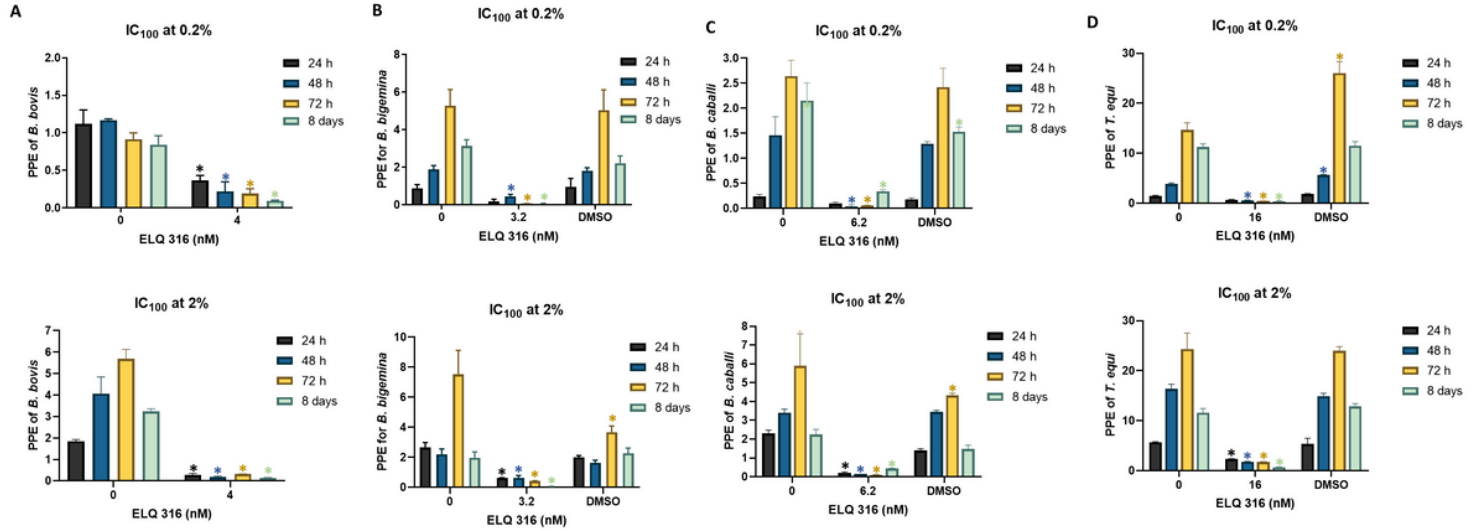

Figure 4

Parasite culture growth at: 24 h (purple bars), 48 h (blue bars), and 72 h (yellow bars) using IC100 of ELQ-316, and 8 days (green bars). (A) B. bovis. (B) B. bigemina. (C) B. caballi. (D) T. equi. Upper panel represents 0.2% PPE and lower panel represents 2% PPE. “0” represents parasites grown without addition of ELQ-316. Assays were carried out in triplicate and the error bars indicate standard error deviation. (*)

Represents P-value <0.05, indicating statistically significant difference between cultures grown without and with ELQ-300 using Student's t-test.

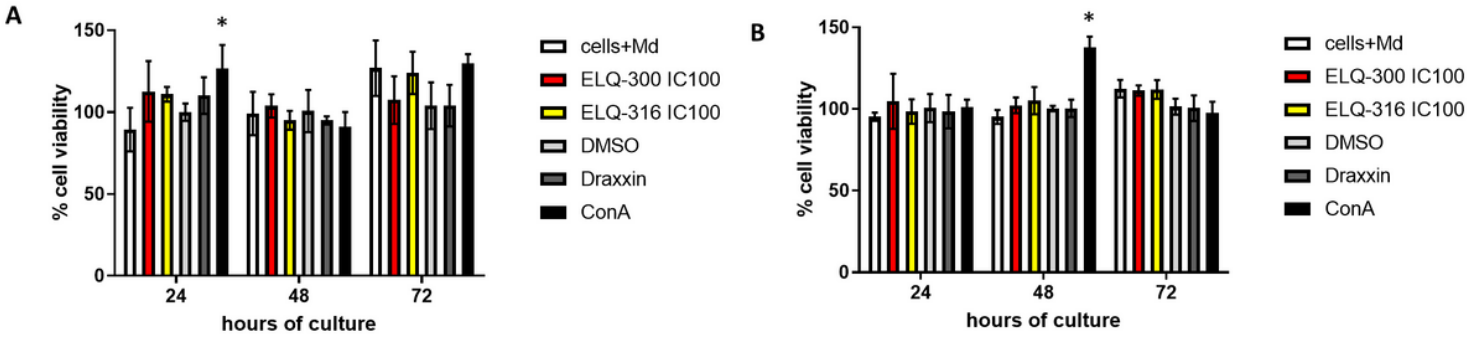

Figure 5

Percentage of cell viability over a period of 72 h after incubation with IC50 and IC100 concentration of ELQs 300 and 316. "Cells+Med" represents PBMC cultivated without addition of the ELQ compounds. Cells in DMSO and Draxxin® were used as a negative control. ConA was used as a positive control for cell proliferation. (A) Bovine PBMC. (B) Horse PBMC. (*) Represents P-value <0.05 indicating statistically significant differences compared to PBMC cultivated in medium only by using Student's t-test. Bovine and horse PBMC were assayed in triplicate and the error bars indicate standard error deviation.

Supplementary Files

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