CFAES

Food Animal Health Research Program, Department of Veterinary Preventive Medicine

Novel Anti-virulence Compound to control Avian Pathogenic *Escherichia coli* (APEC) Infections in Poultry

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Introduction

- Colibacillosis is an endemic disease of poultry caused by avian pathogenic *E. coli* (APEC)
- Colibacillosis is associated with a wide range of symptoms including, airsacculitis, peritonitis, colisepticemia, etc.(1).
- The most prevalent APEC sero-groups causing colibacillosis are O1:K1, O2:K1 and O78:K80.
- The disease is widely prevalent (up to 36.73%) in all age groups of chickens and characterized by high morbidity and mortality. In addition, the disease results in egg and meat production losses. Therefore, the control of APEC is crucial for sustainable poultry farming and food security.
- Current control methods (vaccines and antibiotics) have limited effect, therefore there is a need for novel therapeutic approaches.
- Inhibition of quorum sensing (QS) and virulence mechanisms makes APEC non-virulent similar to a vaccine strain.
- QS is implicated in several physiological state changes including those relevant to biofilm formation, virulence and antibiotic production.
- These quorum sensing inhibitors (QSIs) do not affect APEC growth, making them good candidates to limit the development of bacterial resistance.
- QSI characterize by :
  1) have small size allows them to diffuse readily into cells.
  2) stable and suitable for mass application.
  3) their novel mode-of-action reduces the risk of the development of resistance.

Aims

1. Identify novel approaches to control APEC in poultry
2. Test and evaluate the efficacy of the identified QSIs in APEC-infected chickens

Methods

Activity 1: Testing the best QSIs in infected chickens

- We previously screened a library **4,182** SMs and identified **10** SMs inhibited QS of APEC O78. These 10 QSI possessed no toxicity to chicken RBCs, Caco-2 and HD-11 cells and wax moth larvae and were effective against APEC in Caco-2, HD11 and THP-1 cells and wax moth infected larvae (2).
- **Seven** QSIs (QSI-1, 2, 5-8, and QSI-10) were tested in one-week-old broiler chickens infected with APEC O78 (**Fig. 1**).

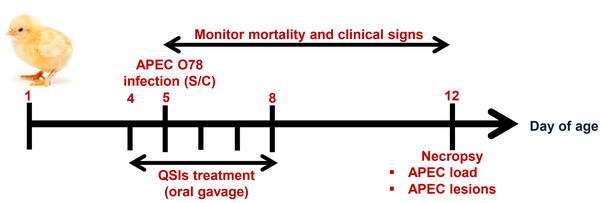


Figure 1. Experimental design for determining the effect of QSIs (QSI-1, QSI-2, QSI5-8, and QSI-10) in APEC infected chickens. QSIs (1 mg/kg body weight) were administered once daily for 5 days (day 4 to day 8) orally. Chickens (n=6/group) were infected subcutaneously (S/C) with rifampicin resistant (Rif^r) APEC O78 (1 x 10^7 CFU/chicken) at day 5. Mortality and clinical signs were recorded from day 5 to day 12. The remaining chickens were euthanized and necropsied at day 12 to determine the APEC load in heart, liver, lung, and kidney and APEC lesions severity in heart, liver, lung, and airsacs. APEC load was quantified using MacConkey agar plates supplemented with 50 µg/ml rifampicin and APEC lesions were scored as described previously(3). Infected non-treated (PC) and non-infected non-treated (NC) chickens were included as controls.

- Effect of the best QSIs (QSI-5, QSI-8, QSI-10) on the gut microbiota of chickens was analyzed by 16S RNA sequencing using QIIME 2 by comparing the relative abundance of microbial community (**Fig. 5**).

Activity 2: Optimizing the dose of QSI-5

QSI-5 possessed the highest efficacy against APEC O78 when given orally. To optimize the dose for drinking water administration, APEC infected chickens were given QSI-5 at doses of 1 mg/L, 5 mg/L,10 mg/L, and 20 mg/L in drinking water (**Fig. 2**).

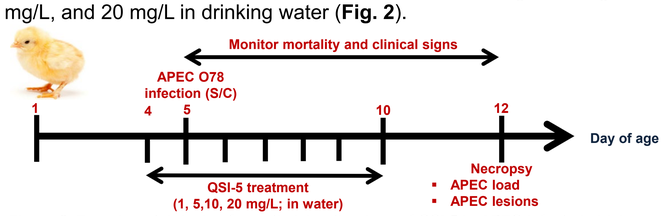


Figure 2. Experimental design for optimizing the dose of QSI-5 in APEC infected chickens. QSI-5 at different doses was administered once daily for 7 days (day 4 to day 10) in water. Chickens (n= 10/group) were infected S/C with rifampicin resistant (Rif^r) APEC O78 (1 x 10^7 CFU/chicken) at day 5. Determination of mortality, APEC load and pathological lesion severity in internal organs was performed as described in activity 1.

Activity 3: Comparing the efficacy of QSI-5 to antibiotic sulfadimethoxine in field simulated conditions

The efficacy of QSI-5 (1 mg/L) to treat APEC infected chickens was compared to the currently used antibiotic (sulfadimethoxine; 495 mg/L) in field simulated conditions (**Fig. 3**).

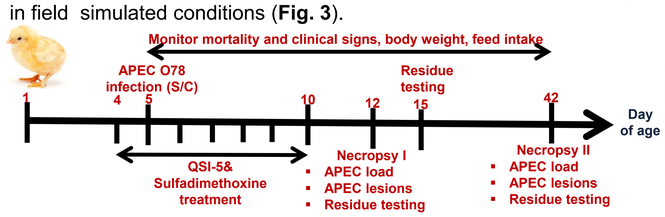


Figure 3. Experimental design for comparing the efficacy of QSI-5 to sulfadimethoxine in APEC infected chickens. QSIs (1 mg/L) and sulfadimethoxine (495 mg/L) were administered once daily for 7 days (day 4 to day 10) in water. Chickens (n= 115) were infected S/C with rifampicin resistant (Rif^r) APEC O78 (1x 10^7 CFU/chicken) at day 5. Necropsy was performed at day 12 (for 30 chickens) and day 42 (for 10 chickens; slaughter age). Determination of mortality, APEC load and lesion severity in internal organs was performed as described before in activity 1. Plasma was collected from chickens (n=5) at 0, 0.5, 1, 2, 4, 8, 12, 24, 84 and 180 h post-administration of QSI-5 and sulfadimethoxine to study their pharmacokinetic (PK) profiles. Five chickens were necropsied at day 2, 5 post treatment (DPT), and at day 42 for testing drug residues.

Results

Activity 1: QSI-5, QSI-8 and QSI-10 significantly reduced chicken's mortality, APEC load and lesions severity in the internal organs.

- **QSI-5 reduced mortality by 100%; while QSI-10 and QSI-8 reduced the mortality by 75% and 50%, respectively compared to PC.**
- **The average reduction of APEC load in internal organs ranged between (5.2- 6.1), (3.4- 4.3) and (2.2-3.1) log CFU/g of tissue in QSI-5, QSI-10 and QSI-8 treated chickens, respectively (Fig 4A).**
- **QSI-5, QSI-10, and QSI-8 reduced the pathological lesions severity in internal organs by (82%- 93%), (85%-100%), and (75%-83%), respectively compared to PC (*P<0.05;* Fig 4B; Table 1).**

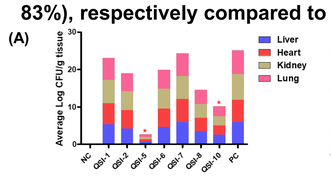
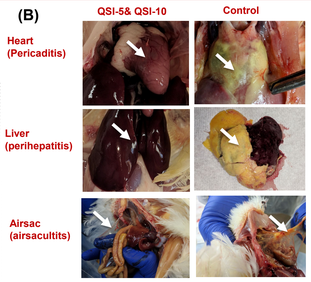


Figure 4A. APEC load in QSIs-treated chickens and PC group. APEC load was quantified in liver, heart, lung and kidney of chickens and presented as average APEC load in all organs of each chicken. *$P<0.05$,One-way ANNOVA.



Scoring was performed as following; **pericarditis;** Normal (0), Vascularization, opacity (1), Cloudy fluid in the pericardial cavity (2), Thickened pericardium (3). **perihepatitis;** Normal (0), Slight amounts of fibrinous exudates (1), Marked perihepatitis with high amounts of fibrinous exudates (2), and Fibrinous exudate accumulate around the liver and make thick layer around the liver (3). **Airsaculitis**; Normal (0), Slight thickened membranes (1), Moderate thickened membranes (2), Severe opaque and thickened membranes (3)

Figure 4B. Lesion scoring in heart, liver and airsac of APEC infected chicken after treatment with QSIs (**representative pictures**). QS-5 and QSI-10 treated chickens showed lesions similar to NC in all organs.

	Liver	heart	airsac	lung
QSI-1	0	0	27	67
QSI-2	45	37	62	89
QSI-5	82	88	93	89
QSI-6	55	44	53	67
QSI-7	33	33	33	83
QSI-8	75	75	67	83
QSI-10	100	85	89	100

Table 1: Pathological lesion scoring (%) of internal organs (liver, heart, lung, and airsac) in QSIs treated chickens compared to PC. The lesions severity in PC control group was normalized to 100%.

- **QSI-5 did not impact the relative abundance of the gut microbiota; while QSI-10 and QSI-8 significantly increased lactobacillus and Butyricicoccus, respectively (*P<0.05;* Fig. 5).**

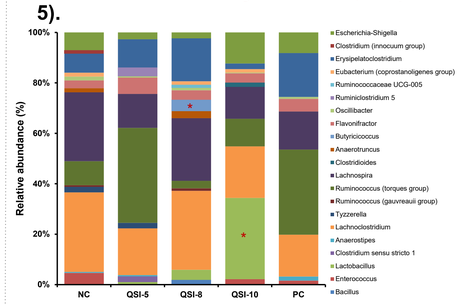


Figure 5. Relative abundance (%) of gut microbes (genus level) in QSIs treated, NC and PC groups. Analysis of microbial sequences was performed using QIIME 2. *$P<0.05$, Wilcoxon each pair test.

Activity 2: QSI-5 at 1mg/L was optimal to treat APEC infection in chickens.

- **QSI-5 at 1 mg/L reduced chicken's mortality by 58%, APEC load in internal organs up to 3.1 logs and lesions severity up to 66.3% compared to PC group (Table 2).**

Table 2: Optimizing the dose of QSI-5 by testing different doses in infected chickens.

	Mortality reduction	APEC load reduction (Log CFU/g tissues)				Pathological lesions reduction (%)			
	(%)	Liver	Lung	Heart	Kidney	Liver	Lung	Heart	Airsacs
1 mg/L	58	3.1	2.3	2.7	2.8	40.8	66.3	41.4	55.8
5 mg/L	25	2.4	2.1	2.3	1.7	36.8	70	32	57.1
10 mg/L	50	2.6	1.6	1	1.6	0	30	12	25
20 mg/L	25	1.9	1.4	1.7	1.3	42.1	60	24	46.4

Activity 3: QSI-5 showed better efficacy in chickens raised on built-up floor litter than currently used antibiotic (sulfadimethoxine).

- **QSI-5 reduced chicken's mortality by 72.2% compared to 35.9% reduction by sulfadimethoxine.**
- **QSI-5 reduced lesions severity up to 88.4%, and APEC load up to 2.8 logs, which was more than the reduction caused by sulfadimethoxine treatment (up to 36.9% of lesions severity, and up to 2.5 logs of APEC load) (Fig 6A; 6B).**
- **QSI-5 released to chicken's blood after 0.5h and reached the peak concentration after 8h; while sulfadimethoxine released after 2h and reached the peak concentration after 24 h.**
- **QSI-5 left no residues in the muscle, liver and kidney of treated chickens; while sulfadimethoxine residues ranged between (0.15-0.99 ppm) and (0.02-0.09 ppm) at 2 DPT and 5 DPT, respectively with no drug residue at 42 days.**
- **QSI-5 treated chickens possessed similar body weight gain and feed conversion ratio compared to NC group.**

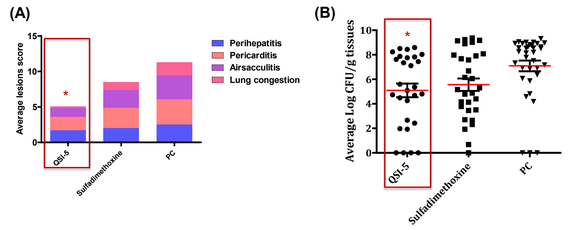


Figure 6. A) average lesion severity in heart, liver, lung and airsacs, and **B)** APEC load in liver, heart, lung and kidney of chickens of QSI-5 and sulfadimethoxine treated groups compared to PC. The data were presented as cumulative average APEC load and lesion severity in all organs of each chicken.

Conclusion

1. QSI-5 possessed the highest efficacy in APEC infected chickens compared to other QSIs.
2. The optimal dose of QSI-5 to treat infected chickens was 1 mg/L.
3. QSI-5 showed no impact on body weight gain, feed conversion ratio and gut microbiota;
4. QSI-5 was detected in 0.5 in blood and no residues in muscle liver and kidney of treated chickens were detected at xx days post treatment.
5. QSI-5 showed better efficacy than currently used antibiotic sulfadimethoxine in field simulated conditions.

- **In summary**, QSI-5 displayed promising antimicrobial properties for the future therapeutic development to control APEC infection in poultry.

Future directions

- Identifying the antibacterial target of QSI-5

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