An exploratory study to characterize current oral fluid usage in pig production systems in the United States

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Industry Summary:
Oral fluid sampling has been widely used in the United States (U.S.) swine industry for at least a decade. However, it is currently unknown how widespread is its use within and across production systems or veterinary clinics. To fill this knowledge, we conducted a pilot study with the aim to characterize the current use of oral fluids sampling in pig production systems in the United States swine industry. We generated two online questionnaires and distributed to production systems and U.S. swine veterinarians during June 2023 to October 2023. Responses from 74 (7 surveys were not complete) surveys were collected, summarized, and analyzed for insights.

Key Findings:

- Widespread Adoption: Almost all (66 out of 67) respondents from 42 companies and clinics are familiar with or actively use oral fluid sampling for diagnostics, indicating its extensive use in the industry.

- Primary Uses: The primary use of oral fluid samples is for routine surveillance (62%) followed by diagnosing clinical cases (33%).

- Sampling Frequency: When oral fluids samples are collected for routine surveillance, 35% respondents collected monthly, 27% collected weekly, and 3% collected daily. In the presence of clinical signs, 61% of respondents increased the sampling frequency, while 22% maintained it at the same level whereas 13% reduced frequency or sampled once.

- Sample Collection Methods: Respondents varied in the number of ropes used per sampled pen, with 69% using one rope for every two pens. The average number of ropes per barn was 3.37 (Interquartile range: 1.75; 4). The average length of time pigs have access to the rope was 25 minutes (Interquartile range: 17.5, 30).

- Sample Management Protocols: 71% of respondents reported having written protocols for sample handling. Half of the respondents have a copy available to farm personnel.

The findings involving 42 companies and clinics, highlight the widespread adoption of oral fluid sampling and its adaptability for routine surveillance and diagnosing clinical cases. The variation in sample collection methods indicates a need for standardization. The study provides benchmarks for the characteristics of current sampling (i.e., frequency, sample size among others) protocols and sample handling/processing and submission protocols.

This research offers valuable insights to producers and veterinarians, enabling them to refine disease monitoring strategies. The information provided in this study reflects how oral fluids are used and their role in surveillance, which will help the industry to better understand how this sampling methodology is applied. The versatility and widespread adoption of oral fluid sampling make it a crucial tool in swine health management. Standardization efforts, based on these findings, will enhance the industry's ability to promptly address emerging diseases and maintain healthy pig populations.

Keywords: oral fluid, sampling, questionnaire, swine health monitoring, sample size, sample frequency, pig production systems

Scientific Abstract:

Introduction:
Oral fluid sampling has been widely used in the United States (U.S.) swine industry for at least a decade. However, it is currently unknown how widespread is its use within and across production systems or veterinary clinics. Furthermore, information regarding sampling characteristics, sample management and whether is part of an active or passive surveillance program is unknown. The aim to characterize the current use of oral fluids sampling in pig production systems in the United States swine industry.

Methods:
Two online questionnaires were employed in two research phases: one targeting field personnel within production systems and another focusing on swine veterinarians. The questionnaires gathered data on the implementation, primary use, sampling protocols, sample handling and processing procedures of oral fluids. A convenient sample of production systems was invited to participate, and the questionnaires were distributed via email through June to October 2023. The collected survey data were cleaned and analyzed using descriptive statistics.

Results:
We received a total of 67 out of 74 valid responses from production systems and veterinarians from 42 companies and clinics. The findings demonstrated widespread familiarity and adoption of oral fluid sampling in the industry, with 99% of respondents reporting its use for diagnostics or surveillance. Primary usage included routine surveillance (62%) and diagnosing clinical cases (33%). The sampling frequency for routine surveillance is monthly in 35% respondents, weekly in 27% cases, and daily in 3% cases. Notably, the sampling frequency increased when clinical signs were present in sites with routine oral fluids sampling. When hanging the rope in the sampled pen, 69% use one rope for every two pens, 21% use one rope per sampled pen. The mean number ropes used per barn was 3.37 (Interquartile range, IQR: 1.75; 4) and the mean number of pigs in the airspace per rope was 492 (IQR: 275; 600). The mean number of pens sampled per barn was 8 (IQR: 4; 9) and mean number of pigs represented by a single oral fluid-rope sample being 214 (IQR 60; 275). Most respondents reported having written protocols for sample handling, with 50% making these protocols available to farm personnel.

Discussion:
This study offers a comprehensive overview of oral fluid usage in U.S. pig production systems. It underscores the need for standardization in collection methods to further advance the utilization of oral fluid sampling in veterinary practice. The information provided in this study reflects the oral fluids sample management and its role in surveillance, which will substantially push forward the standardization of the oral fluids collection and handling, aid in the early detection of emerging pathogens, and improve swine health monitoring.

Keywords: oral fluid, sampling, questionnaire, swine health monitoring, sample size, sample frequency, pig production systems

Introduction: Oral fluid sampling has been widely used in the United States (U.S.) swine industry for at least a decade. This sample type has been rapidly adopted by the industry for endemic disease monitoring as it readily optimizes time and resources when conducting population-based-like sampling. Furthermore, the fact that different assays have been and will continue to be developed to detect either pathogens or antibodies in saliva makes this sampling approach useful for veterinarians and producers as the specimen collected in the same sampling event can be used to test for different pathogens or antibodies. Different studies have been conducted to assess the sensitivity of this sample type for specific pathogens and guidelines for sampling have been proposed. However, even though oral fluids are a widely accepted sampling methodology, it is currently unknown how widespread is its use within and across production systems or veterinary clinics. Furthermore, information regarding sampling characteristics, sample management and whether is part of an active or passive surveillance program is unknown. Information regarding how pig production systems and practitioners are using this sampling methodology is important to better understand how this sampling methodology aid in the early detection of emerging pathogens.

Objectives: This pilot project aimed to characterize the current use of oral fluids sampling in pig production systems in the United States swine industry.

Specific objectives:
a) Determine whether oral fluids are being implemented and what is their primary use
b) Characterize current sampling (i.e., frequency, sample size among others) protocols
c) Characterize sample handling/processing and submission protocols

Materials & Methods:
Questionnaire generation

We generated two online questionnaires (Questionnaire #1, Questionnaire #2) via Qualtrics for the two research phases in this project. Two questionnaires were built with Qualtrics, in which a variety of multiple-choice together with open-ended questions were included to obtain the data requested by CEAH-NPB. The questionnaires were shared with company and private practice veterinarians, production managers and site managers. In the first phase, conveniently selected production systems were invited to fill out Questionnaire #1 regarding oral fluids usage, which was expected to deliver the field insights. In the second phase, U.S. swine veterinarians were invited to take Questionnaire #2 which was expected to increase the representativeness of the data. Both questionnaires have the same content structure, with slight differences considering the difference in targeted groups. Both questionnaires consisted of five sections, the content of the questionnaire is summarized in Table 1.

Table 1. Content structure of two questionnaires on oral fluids usage in pig production systems in the United State

Sections	Details	Questionnaire #1	Questionnaire #2
Demographics	Name and contact information	X	X
	Respondent role (e.g., producer, veterinarian, contract grower, supervisor)	X	X
	Affiliated companies/clinics and job title	X	X
Information related to the pigs they oversee	Number of sows and growing pigs they are responsible for	X	X
	Number of sites, barns at site, pigs per barn, pens per barn, pigs per pen, and farm type for which they are responsible	X	
Oral Fluids Usage Information	Are they familiar with oral fluids collection and its use for diagnostics	X	
	Do they use oral fluids for diagnostics		X
	Frequency of sampling - routine	X	
	Frequency of sampling per farm type - routine		X
	Frequency of sampling - no clinical sign		X
	Frequency of sampling - with clinical sign, whether other factors impact sampling frequency)	X	X

	Primary use of oral fluids as a diagnostic sample		X	X
	Sample size	No. ropes per barn when collecting oral fluids	X	
		No. ropes per barn and pigs when collecting oral fluids for routine surveillance		X
		No. ropes per barn and pigs when collecting oral fluids based on presence of clinical signs		X
		Method to determine the number of ropes to hang	X	X
		Average No. ropes used per sampled pen	X	X
		Average No. pens sampled per barn	X	X
		Average No. pigs represented by a single oral fluid-rope sample	X	X
Sampling and Sample Management Procedures	Sampling	Personnel responsible for oral fluids sample collection	X	X
		Length of time pigs have access to the rope	X	X
		Source and the diameter of the rope	X	X
		Availability of written protocol for handling/processing the ropes	X	X
	Storage and shipping process	Storage of collected sample	X	X
		Time range between collecting the oral fluids and submission to the diagnostic laboratory	X	X
Sample submission	Where the samples go next after collection		X	X
	Information includes when oral fluids samples are submitted		X	X

Questionnaire distribution

We invited a convenient sample of pig production systems for the first research phase (Questionnaire #1). As a starting point we invited three production systems accounting for approximately 200K sows and their downstream production. We reached out to the company veterinarian and explained the objective of the project and explored the possibility of distributing the questionnaire internally so that field personnel, which are the ones collecting the samples, contribute to data gathering and thus help us obtain quality data. For the second research phase, we targeted swine practitioners by a) contacting all Morrison Swine Health Monitoring Project (MSHMP) participating veterinarians and b) inviting U.S. based American Association of Swine Veterinarians (AASV) members. The questionnaires links were shared via email, the link for Questionnaire #1 was valid from June 7, 2023 to October 19, 2023, the link for Questionnaire #2 was valid from September 1, 2023 to October 19, 2023. A $25 gift card was offered to the first 100 respondents.

Data cleaning and analysis

The collected survey data was converted into MS Excel spreadsheet. Since most of the content of the two questionnaires is consistent, the survey data of the two questionnaires were combined, and the few different questions were analyzed separately. The survey data was cleaned by manually check and incomplete or duplicated responses were removed from the further data analysis. Descriptive statistics were performed on the cleaned survey data. T-test were conducted in R to determine if there was a significant difference between the means of the two groups (R Core Team, 2023).

Results:

Key findings

- Widespread Adoption: almost all (66 out of 67) respondents from 42 companies and clinics reported that "they are familiar with oral fluids collection and its use for diagnostics" or "use oral fluids for diagnostics", this indicates the widespread use of oral fluids for diagnostic in pig production systems. A total of 7 surveys were removed from the database given that these were not complete.

- Primary Use: the primary use of collected oral fluids samples was mainly for routine surveillance (62%) and diagnosis when clinical signs are present (33%).

- Sampling Frequency: A total of 35%, 27% and 3% of the respondents collected oral fluids on a monthly, weekly and daily basis respectively.

- Sampling Frequency with Clinical Signs: when clinical signs are present and compared to when clinical signs are absent, the oral fluids sampling frequency increased in 61% cases (i.e., changed from monthly to weekly or from weekly to daily); in 22% cases, the oral fluids sampling frequency stayed the same regardless clinical signs present; 13% of the respondents decided to decrease the frequency or just sample once when clinical signs arise.

- Sample Collection Methods: for the number of ropes hung per sampled pen, 69% of the respondents use one rope for every two pens, 21% use one rope per sampled pen, while the remaining respondents employ alternative approaches, such as using three ropes per barn, two ropes per barn, or making determinations based on airspace conditions.

- Sample Size: the mean number of ropes per barn was 3.37 (IQR 1.75; 4); Mean number of pens sampled per barn was 8 (IQR: 4; 9); Mean No. pigs represented by a single oral fluid-rope sample: 214 (IQR 60; 275). The average length of time the pigs have access to the rope was 25 minutes (Interquartile range: 17.5, 30).

- Sample Management Protocols: the most (71%) respondents reported they have written protocol for handling/ processing the ropes available, in 50% cases the farm personnel have a copy of the protocol.

Complete results

In the first research phase, Questionnaire #1 was sent to three pig production systems, yielding responses from 8 participants. In the second research phase, Questionnaire #2 was distributed to veterinarians, eliciting responses from 59 participants. After survey data combining and cleaning, we received a total of 67 valid responses for the two questionnaires.

Demographics and related pig information

Among 67 respondents from 42 production companies or clinics, 91% were veterinarians and 9% were supervisors including 5 serviceman/service manager from nursery and finishing sites, and 1 gilt development manager. Based on the surveys, respondents account for 58 million growing pigs and 3.9 million sows; however, we believe these populations may be overestimated due to overlapping between company and clinic responses. More detailed pig population information was collected in Questionnaire #1. The average number of sites the respondents are responsible for was 22 (Median: 11, IQR: 7; 13). Table 2 represents information from the 70 sites represented in Questionnaire #1, consisting of 34 (49%) nurseries, 34 (49%) finishers, 1 wean to finish, and 1 wean to market site.

Table 2. Pig population information collected in Questionnaire #1, based on responses from eight respondents.

Farm type (No. sites)	No. Pigs / Barn Mean [IQR]	No. barns.at the site Mean [IQR]	No. Pens / Barn Mean [IQR]	No. pigs /pen Mean [IQR]
Finisher (34)	2333 [1200; 3000]	2 [1; 2]	43 [20; 76]	57 [33; 82]
Nursery (34)	3712 [2200; 5100]	1 [1; 2]	111 [80; 157]	53 [34; 40]
Wean to Finish (1)	2400	1	100	25
Wean to Market (1)	4900	2	40	122

Objective 1: Oral fluids implementation and its primary use

Almost all (99%) respondents stated that "they are familiar with oral fluids collection and its use for diagnostics" or "use oral fluids for diagnostics", this indicates the widespread use of oral fluids for diagnostic in pig production systems.

In general, the primary use of oral fluids as a diagnostic sample was mostly (62%) for routine surveillance (regardless of clinical signs), followed by 33% based on the presence of clinical signs (e.g., cough, sneeze, diarrhea, mortality), 3% pig shipments, and 2% for both surveillance and clinical signs.

Objective 2: Current sampling protocols

Frequency

The frequency of oral fluids collection for routine surveillance is summarized in Table 3. When the respondents were asked to summarize the sampling frequency for routine surveillance according to the farm type, we received 162 answers. The reasons for receiving 162 answers are: a) The questionnaire includes three farm types (i.e., GDU, Sow farm and Growing pig farm), ideally each respondent would answer the sampling frequency for the three farm types separately; however, some respondents only filled in the types of farms they work with rather than the three types. b) The same farm type was applied for more than one sampling frequency, e.g., GDU was sampled both weekly and monthly in some responses. Among these answers, 35% collected oral fluids monthly, 27% collected oral fluids weekly, and only 3% collected oral fluids daily. The other collecting frequency was described in Figure 1. Table 3 and Figure 1 together show that among all site types, the most frequent collection of oral fluids for routine monitoring (e.g., daily, weekly, monthly) is GDU.

Table 3. The percentage of different farm types' oral fluids sampling frequency for routine surveillance

Frequency of sampling -Routine	Farm Type				Percentage
	GDU	Sow Farm	Growing Pig Farm	Not specified in Q1*	
Daily		1%	2%		3%
Weekly	12%	2%	12%	1%	27%
Monthly	19%	9%	6%	1%	35%
Other (See more details in Figure 1)	6%	12%	14%	3%	35%

* The farm type was not specified in Questionnaire #1 for the question on the frequency of oral fluids collection for routine surveillance.

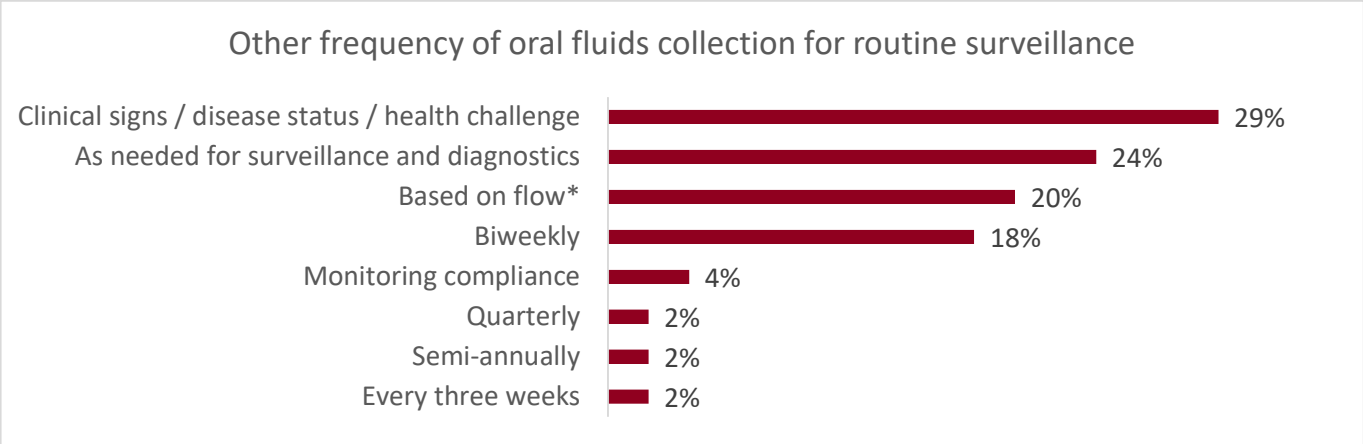

Figure 1. Survey results on other frequency of oral fluids collection for routine surveillance.
*Examples on oral fluids collection based on events related to pig transport and flow: collect when gilts are being shipped; on arrival and at end of quarantine in GDU, halfway through the turn and prior to shipment, post arrival testing in the ISO 2 days and 7 days post arrival, GDU 2-4 days post arrival and 4-5 days before entry to sow farm)

Figure 2 shows the frequency of oral fluid sampling with and without clinical signs. When there are clinical signs, the frequency of oral fluids collection varies (Figure 2A). On the one hand, when clinical signs begin, sites

collected oral fluids more frequently on a weekly or daily basis compared with routine surveillance (Figure 2B). On the other hand, some sites collect oral fluids only once for disease diagnosis and find that there is little need to continue sampling and testing. When there are no clinical signs (Figure 2B), the collection frequency is mainly determined by other factors (e.g., animal type, flow, clinical signs, pig density and disease pressure) which accounted for 30% of the responses. We specifically looked at the responses reporting a regular monitoring when there are no clinical signs, compared to oral fluids sampling frequency when there are clinical signs on the site (Figure 3). The sampling frequency increased in 61% cases (i.e., changed from monthly to weekly or from weekly to daily when clinical signs present). In 22% of the cases, the oral fluid sampling frequency remained the same regardless of whether clinical signs were present whereas 13% of the respondents decided to decrease the frequency or just sample once when clinical signs arise.

Further, 65 of the 66 respondents who collected oral fluids agreed that the oral fluids sample frequency is timed on other factors, and 1 said they stick to a weekly basis. The most mentioned other influencing factors were pre-shipping sampling (23%) and oral fluids collection after new pig arrival (23%), followed by surveillance for different site type (16%), and suspect IAV, PRRS, PED sites / clinical signs and diagnosis (16%). Disease pressure in the area was also mentioned in 7% of the listed concerns. The impact of disease pressure may vary as one voice was that PRRS in the area will designate more frequent testing and another voice was that in clean areas the sites will be tested more frequently, with sites in PRRS areas testing less frequently. Other listed factors were location of the site near to a sow farm or boar stud, pig movement (not specified), sow farm status, current site status, and risk to other farms.

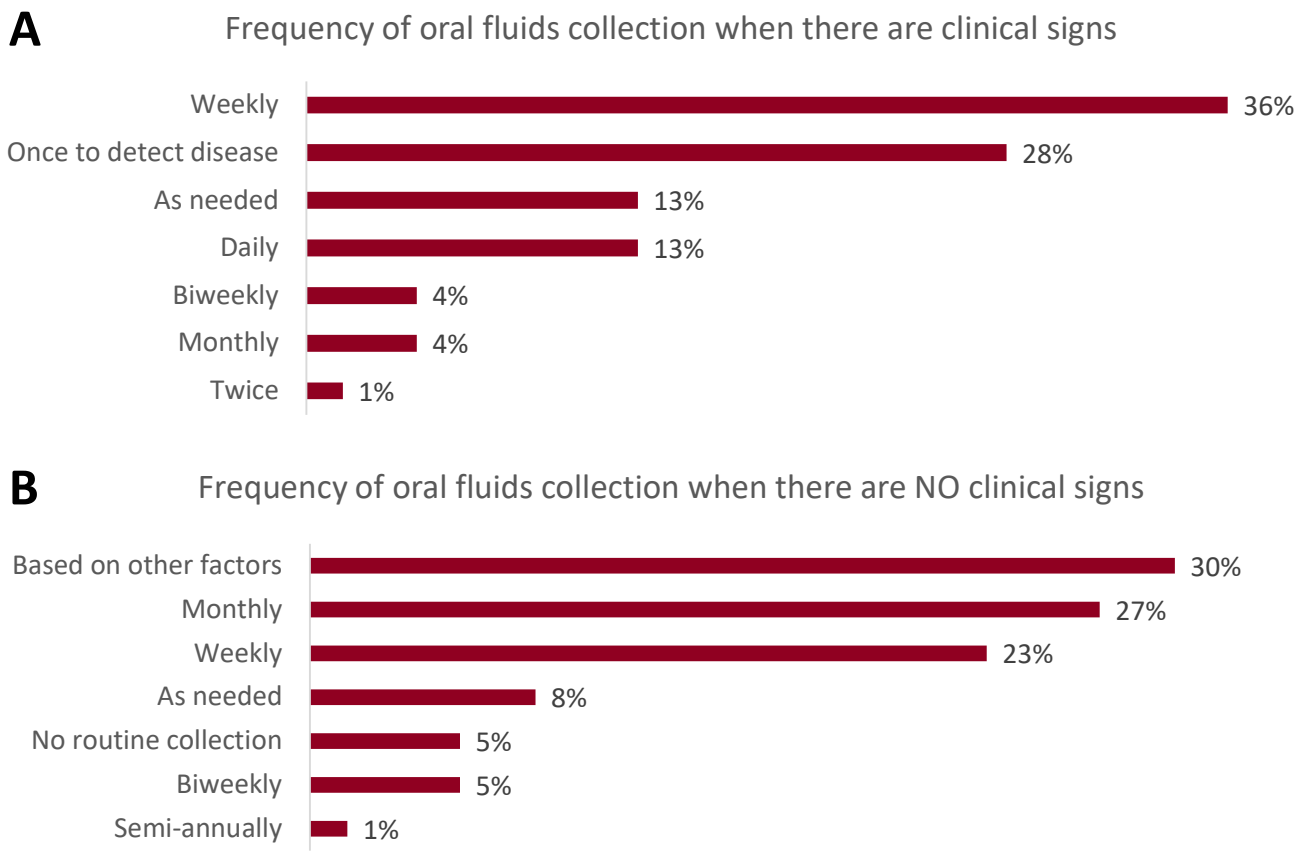

Figure 2. Survey results on the frequency of oral fluids collection when there are clinical signs (2A), and when there are no clinical signs (2B).

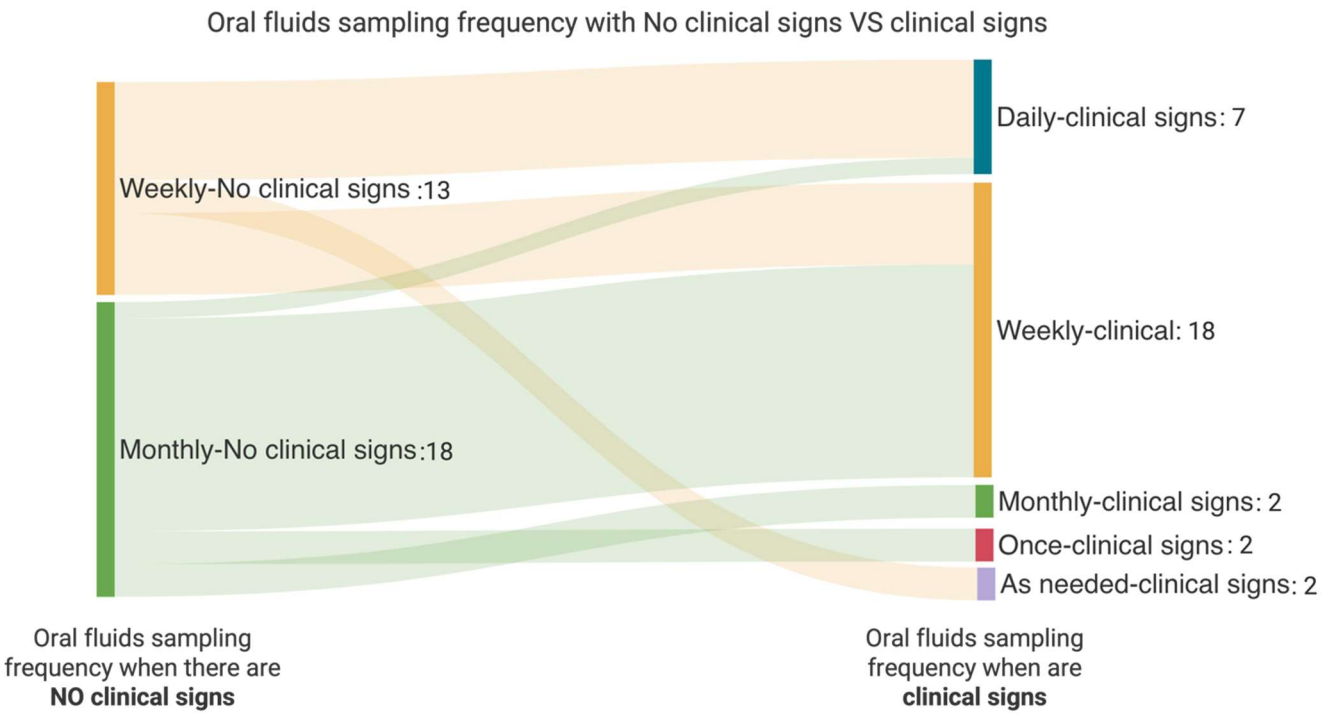

Figure 3. Oral fluids sampling frequency changes with and without clinical signs on site.

Sample size

For the number of ropes used per sampled pen, the majority of respondents (69%) use one rope for every two pens, 21% use one rope per sampled pen, while the remaining respondents employ alternative approaches, such as using three ropes per barn, two ropes per barn, or making determinations based on airspace conditions.

At the barn level, the mean number of ropes per barn was 3.37, with a median of 3 and an IQR of 1.75 to 4 (Table 4). On average, the number of pens sampled per barn was 8, ranging between 1 and 15. Notably, three complex responses that were not included in the table stated that they test all pens for gilts, which indicates that the number of pens sampled oral fluids per barn would vary based on barn size and layout.

For routine surveillance, 1 rope was hung for on average every 492 pigs in the airspace, the median was 500 pigs. When collecting oral fluids based on presence of clinical signs, 1 rope was hung for on average 535 pigs in the airspace with the same median as the routine sampling. A t-test on the number of pigs per rope in the airspace in the routine and clinical scenarios showed no statistically significant difference ($p= 0.49$). Additionally, the number of pigs represented by a single oral fluid-rope sample had a mean of 214, a median of 150, and ranging from 5 to 1200. Both these results account for situations in which the respondents pool the samples in different ways.

Table 4. Sample size* related to oral fluids collection in pig production systems

	Mean	Median	Min; Max	IQR
Number of ropes per barn	3.37	3	1; 15	1.75; 4
Number of pens sampled per barn	8	6	1; 30	4.25; 9
Number of pigs per rope in the airspace** - routine	492	500	38; 1,100	275; 600
Number of pigs per rope in the airspace** - clinical signs	535	500	38; 2,000	300; 633
Number of pigs represented by a single oral fluid-rope sample**	214	150	5; 1200	60; 275

*Data in this table was collected through open-ended questions, few complex answers could not be converted directly to numbers and are not reflected in this table but described in the text.

** Number of pigs per rope represents one rope was hung in the airspace of how many pigs, and not all pigs in this airspace necessarily have access to the rope, while the number of pigs represented by a single oral fluid-rope sample is the number of pigs that are considered to have chewed the rope.

As for the sample size determination, 54% of the respondents decided the number of ropes needed by themselves, 29% referred to the number dictated by company/clinic guidelines, 9% used the number dictated by their veterinarian, the remaining 8% indicated using multiplication, company/research guidelines or it was related to the nature of disease/site health status.

Personnel responsible for oral fluids sample collection

Among the 66 respondents using oral fluids for diagnosis, in 32 cases (48%) the caretaker collected the sample, 12 respondents (18%) answered that the sample was collected by the veterinarian or the supervisor, 12 (18%) alluded to the regional production supervisor, 5 (8%) to the company/clinic health technician, and the remaining (8%) mentioned field team, service manager together with supervisor, or depending on the needs.

Length of time pigs have access to the rope

The distribution of the length of time pigs have access to the rope is shown in Figure 4. The average length of time pigs have access to the rope was 25 minutes, with a median of 20 minutes (IQR: 17.5, 30). We removed one answer that reported hanging the rope for 24-48 hours. It was also mentioned in two responses that the time pigs have access to the rope depends on the age and size of the pig, finishing pigs will have shorter access to the ropes, otherwise the ropes are lost and cannot be collected.

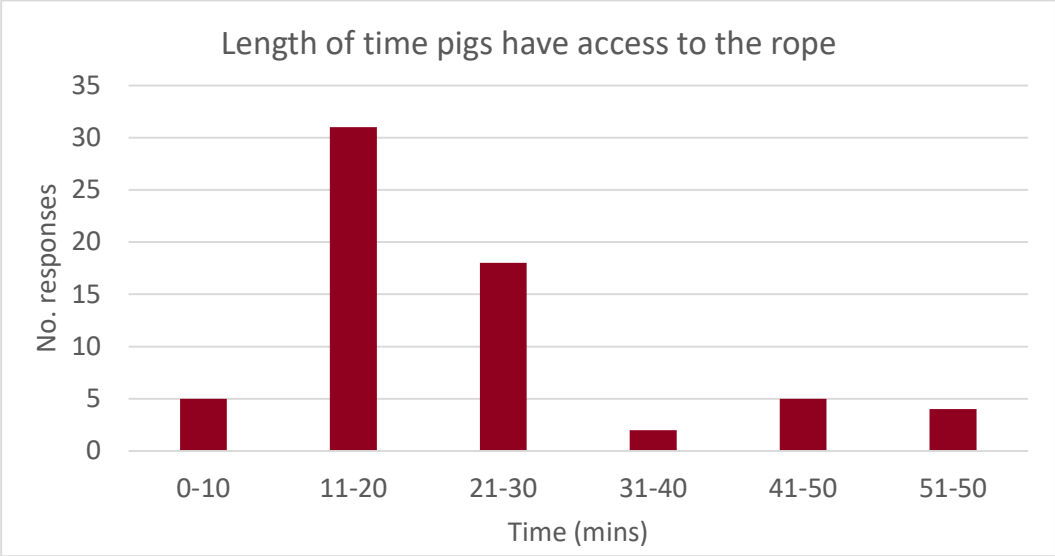

Figure 4. The distribution of responses on the length of time pigs have access to the rope.

Source and the diameter of the rope

The source of the rope was unknown for the majority (55%) of the respondents as shown in Figure 5. The known store/vender varied among responses including Knot & Rope Supply, AHI, Ravenox, etc, so as the online source (Amazon, langmanropes.com, knotandrope.com). Some respondents mentioned their clinic as the source of the rope. The rope diameter was unknown or unspecified in 44% of responses, with the remainder averaging 0.74 inches, median 0.63 inches, and IQR 0.5 to 0.75 inches.

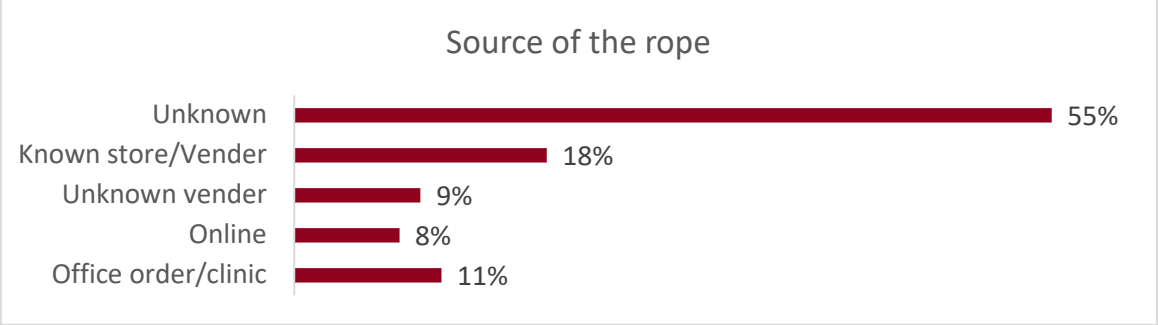

Figure 5. Survey results on the source of the rope based on 66 responses.

Objective 3: Sample handling/processing and submission protocols

Availability of written protocol for handling/processing the ropes

Most (71%) respondents answered they have a written protocol available for handling and processing the ropes and in 50% of those cases the farm personnel have a copy of the protocol (Figure 6). Meanwhile, 29% of the responses have no written protocol to guide the rope handling and processing yet.

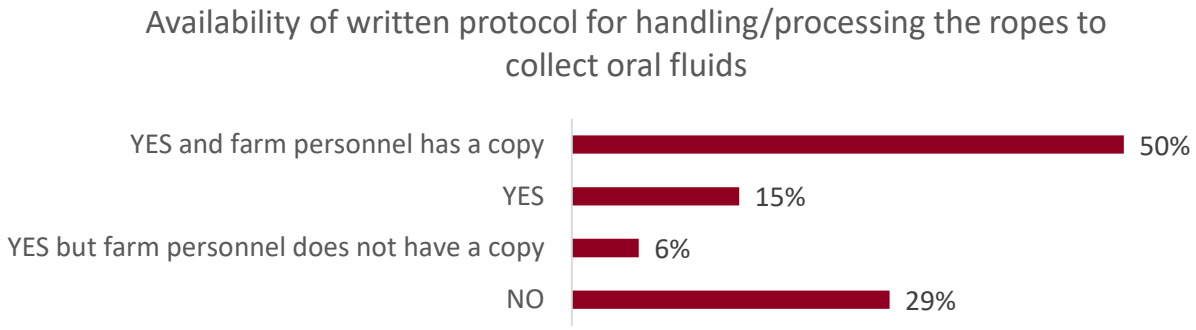

Figure 6. Survey results on the availability of written protocol for handling/processing the ropes.

Storage of collected sample

Once oral fluids samples are collected, 76% of the responses specified that the samples were stored in refrigeration, in which half were stored (38%) at the farm, and the other half (38%) were left in the veterinarian/regional production supervisor truck under refrigeration until they arrive at the office. A total of 8% responses indicated the samples are placed on ice in a cooler and driven or shipped to the lab. Only 9% of the respondents indicated that samples would not be kept refrigerated until they arrived at the office for refrigeration. In 6% of the cases, respondents stated the refrigeration logistics may vary based on the schedule of the day, without specifying the refrigerator situation.

Time range between collecting the oral fluids and submission to the diagnostic laboratory

As for the time that goes by between oral fluids collection and submission to the diagnostic laboratory, 91% of the respondents provided the time range. The descriptive summary is presented in Table 5. The mean average time between sample collection and submission was 20.2 hours, with a range of 2 to 48 hours. The minimum time span ranged between 0 to 48 hours, with an average of 9.4 hours. The maximum time between collection and submission averaged at 42.5 hours, 2 responses commented that the longer hours were due to weekend collection or submission.

Table 5. Descriptive summary of the average, minimum and maximum time between sample collection and submission.

Time between sample collection and submission	Mean	Minimum	Maximum	IQR
Average (hours)	20.2	2	48	12; 24
Minimum (hours)	9.4	0	48	2; 16
Maximum (hours)	42.5	4	96	24; 67

Where do the samples go next after collection?
The processing pathway for collected samples is shown in Figure 7. The majority of the collected samples were first processed at the local lab or clinic (44%) and then shipped to the VDL. In 35% of the cases these were sent directly from the farm to the VDL, while 9% were sent to clinic (5%) or other vet's clinic (5%). Additionally, 3% samples were sent to the production company laboratory.

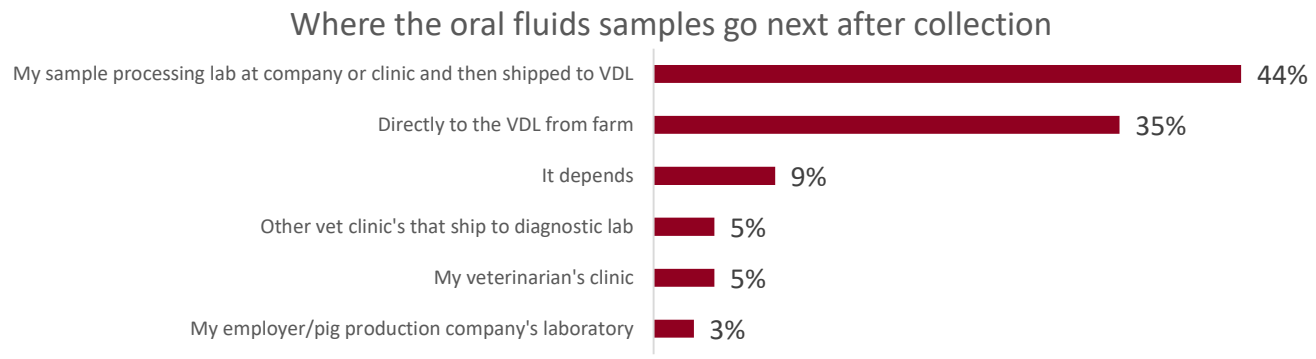

Figure 7. Survey results on where the oral fluids samples go next after collection.

Information included when oral fluids samples are submitted
When oral fluids samples are submitted to the VDL, the information included is summarized in Figure 8. Pig age was included most of the time (91%), followed by the Premises Identification Number (83%) and whether clinical signs were observed (64%). Specific originating pens and barns is also mentioned when submitting oral fluids samples in 47% responses.

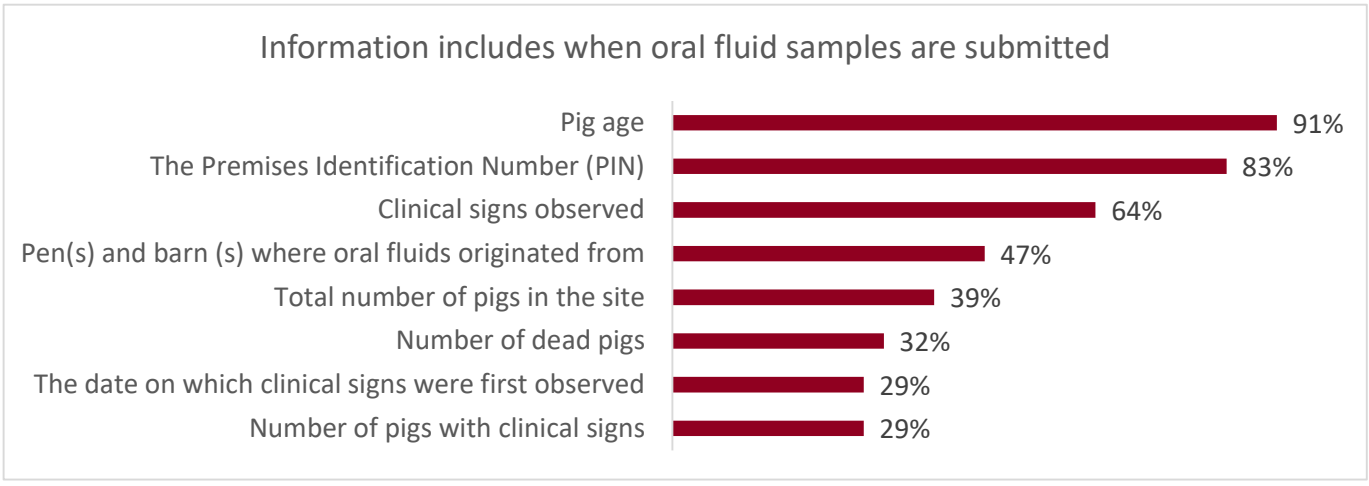

Figure 8. Survey results on the information includes when oral fluids samples are submitted.

Discussion:

Objective 1: Oral fluids implementation their primary use

Veterinarians and producers responded to this survey, with 99% indicating "they are familiar with oral fluids collection and its use for diagnostics" or "use oral fluids for diagnostics". This robust 99% usage rate

highlights the extensive adoption of oral fluid collection for diagnosis in the U.S. pig production. The reported primary use of collected oral fluids samples was mainly for routine surveillance and diagnosis when clinical signs present.

Objective 2: Current sampling protocols

The frequency of oral fluids collection varied mainly based on two aspects, farm type and clinical signs.

1) Farm type. For routine surveillance, the GDU was sampled more often than sow farms and growing pig farms and was collected more on a regular basis than on an as-needed basis. Except for routine monitoring (weekly, biweekly, or monthly), some veterinarians also reported that in GDU they collect oral fluids based on animal movements, e.g., 2-4 days post arrival in the GDU and 4-5 days before entry to sow farm. In sow farms, routine oral fluids test mostly happens monthly, otherwise as needed (Table 3). For example, some veterinarians indicated that sow farms with an isolation facility routinely test the gilts before entering the sow herd, roughly 2-7 days post arrival. The oral fluids samples collection in growing pig farms varies, 33 responses reported they collect the samples routinely, while some veterinarians only collect oral fluids in growing pig farms as needed (e.g., when clinical signs present).

2) Clinical signs. We also looked at the responses reporting routine oral fluids collection when there are no clinical signs. For these cases, when there are clinical signs, the sampling frequency increased in 61% cases, stayed the same in 22% cases, and decreased in 13% responses. This represents differences in farm management decisions and disease control approaches, but generally reflects that farms monitoring oral fluids regularly turn to continue to track infections after the onset of clinical symptoms.

The oral fluids sample size differs based on several factors. 1) Farm type - Fewer pigs per rope in the airspace may be sampled at the GDU than growing pig farms. For instance, a response reported that they hung one rope per 400 grow/finishing pigs, and one rope per pen at GDU facilities. This suggested a further investigation on the oral fluids sample size considering the farm type. 2) Barn designs - The ropes hung per pen was reported to be dependent on barn design. Large pen in finisher (e.g., 250 to 500 pigs) may use two ropes per pen. 3) Clinical signs – Although the t-test on the number of pigs per rope in the airspace in the routine and clinical scenarios showed no statistically significant difference, some respondents did report different rope hanging strategies when there are health threats. In case there are clinical signs present, if the farm is in isolation or need higher sensitivity of detection, the veterinarians will hang one rope per pen instead of one rope per two pens to limit the pooling. On the other hand, given the presence of clinical signs, it may be easier to detect the virus, thus there was a respondent that reported hanging 4 ropes per a 1200 pig-barn for routine surveillance, while this was reduced to 2 per barn when clinical symptoms were present. Therefore, the sampling strategies are dependent on the detection needs (e.g., higher sensitivity, detection the presence of virus).

Data on sample size were collected through open-ended questions in the questionnaire with the goal of obtaining as much information as possible as we anticipated different sampling approaches, so Table 4 does not fully cover all inputs. For example, the range of number of pens sampled per barn ranged between 1 and 15; however, some respondents reported that they sample all gilt pens. Thus, the number of pens sampled per barn may be higher than what has been summarized in Table 4.

The definition of the following two variables is different: Number of pigs per rope per airspace and Number of pigs represented by a single oral fluid-rope sample. The former represents one rope was hung in the airspace of a specific number of pigs, which means not all pigs in this airspace necessarily have access to the rope. On the other hand, the number of pigs represented by a single oral fluid-rope sample is the number of pigs that the respondent believed chewed the rope. Furthermore, both variables account for situations in which the respondents pool the samples in different ways. For example, a respondent reported that they divide two ropes into 6 strands and place those six strands throughout the barn (~1000 pigs) and then they will pool all six strands into one tube. In this case, the number of pigs per rope in the airspace and number of pigs represented by a single oral fluid-rope sample were calculated as 500 and 1000, separately.

Objective 3: Sample handling/processing and submission protocols

Our survey data shows the most (71%) respondents having a written protocol for handling and processing the ropes available, and in 50% of the cases the farm personnel have a copy of the protocol. A total of 84% of the responses indicated proper storage method (i.e., the oral fluids samples were refrigerated or placed on ice in a cooler after

collection) largely demonstrated the compliance with the protocol. A further way to assess the compliance of the protocol would be to validate our collected information (e.g., time range between collecting the oral fluids and submission to the diagnostic laboratory, the path samples go next after collection) to individual protocols. This survey data also suggested that sample submissions provide extra essential information when submitting the oral fluids samples. For example, quantitative information such as the number of pigs with clinical signs, the date on which clinical signs were first observed, number of dead pigs, total number of pigs in the site, pig age. This way will help further within and between farm disease investigation.

The use of oral fluid sampling in pig production systems is still developing, and further standardization of the collection methods is needed to use it regularly in veterinary practice (Ornelas et al., 2023). This project, which covered respondents from 42 companies and clinics, provides a comprehensive overview of the oral fluids usage in U.S. pig production given its high level of representativeness. The information provided in this study reflects the oral fluids sample management and its role in surveillance, which will help the industry to better understand how this sampling methodology is currently being used. This understanding will substantially push forward the standardization of the oral fluids collection and handling, aid in the early detection of emerging pathogens, and improve swine health monitoring.

References

R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Ornelas, M. A. S., López-Martínez, M. J., Franco-Martínez, L., Cerón, J. J., Ortín-Bustillo, A., Rubio, C. P., & Manzanilla, E. G. (2023). Analysing biomarkers in oral fluid from pigs: Influence of collection strategy and age of the pig. *Porcine Health Management*, *9*(1), 39. https://doi.org/10.1186/s40813-023-00333-x