

Water quality in turkey farms in Khemisset (Morocco) and potential risk factors

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Summary

The objectives of this study were to assess the microbiological and physical/chemical quality of water in broiler turkey farms in the province of Khemisset (north-western Morocco) and, based on a questionnaire, to ascertain potential risk factors for contamination of drinking water with faecal coliforms. A total of 80 samples were collected and analysed in 20 farms (4 from each farm). At the main inlet to the water line at the entrance to each turkey house, 100% of the samples were of unacceptable quality in terms of faecal coliforms, *Escherichia coli*, faecal streptococci, sulphite-reducing anaerobes and enterococci. A significant reduction in microbiological contamination of the water line ($p < 0.05$) was observed on Day 60. While more than 90% of the samples were of satisfactory quality in terms of pH, nitrites, conductivity, nitrates and iron, only 35% were satisfactory in terms of total hardness and only 20% met quality standards for ammonium content. The factors affecting levels of contamination with faecal coliforms were water chlorination ($p = 0.065$; odds ratio = 14;

90% confidence interval [CI] = 1.14–71), cleaning and disinfection ($p = 0.028$; odds ratio = 14; 95% CI = 1.25–156.6) and antibiotic treatment ($p = 0.001$; odds ratio = 6; 95% CI = 2.1–35.2).

To improve water quality in poultry farms, farmers are advised to protect wells from contamination and to install water purification units (pre-oxidation, coagulation, flocculation, disinfection). In addition, turkey houses and rearing equipment should be rigorously cleaned and disinfected between each batch of birds.

Keywords

Bacteriological quality – Broiler turkey – Contamination – Drinking water – Morocco – Physical/chemical quality.

Introduction

In Morocco, monitoring of drinking water quality for poultry is a recent concern. For many years, the potential impact of poor-quality drinking water, both on animal health and on the solubility of drugs and their therapeutic efficacy, was underestimated.

Factors influencing water quality are: the presence of bacteria, especially coliform; high or low pH; extreme hardness; high levels of iron, magnesium, nitrates, nitrites, sodium or chlorine; and the presence of other minerals (1).

In poultry farms, water is used at all stages of production. It is used not only for watering animals and cooling buildings, but also for administering drugs and vaccines, and it is essential for cleaning and disinfecting facilities.

This ubiquitous use of water explains why any change in its quantity or quality can adversely affect livestock health and performance (2). A 2004 study in France showed that farms using water of higher bacteriological quality achieved better technical results and that the animals on these farms experienced fewer digestive disorders (3). Several authors (4) report that water quality is a major factor in controlling animal health problems and that farmers are quick to take

action if water quality is poor. Indeed, as several studies have shown (5, 6), the presence of pathogens in drinking water poses a risk to poultry health and performance.

Although a number of physical/chemical studies have been conducted in Morocco (1, 7, 8), until now no study has examined the risk factors for bacterial contamination of poultry drinking water.

The authors therefore undertook this study with two objectives:

- to assess the microbiological and physical/chemical quality of water in broiler turkey farms in the province of Khemisset (north-western Morocco)
- to ascertain potential risk factors for bacterial contamination of drinking water.

Materials and methods

Choice of farm sites

The sites covered by the study are located in the province of Khemisset in Morocco's central plateau region. Khemisset's climate is temperate, semi-continental and perfect for agriculture. These factors have favoured the development of broiler turkey farms in the region. Geographically, Khemisset province is located in north-western Morocco. It comprises 32 rural municipalities across which the 35 broiler turkey farms identified by the agricultural services are distributed, 20 of which were operational at the time of the study.

Sampling

This study was conducted in 2011 on all operational farms ($n = 20$), representing 86 turkey houses. Total production capacity was 439,000 broiler turkeys per batch. One statistical unit was equal to one house per farm.

Four water samples were collected from each farm in 500-ml sterile bottles, using a specific procedure to prevent any accidental contamination. They were then transported to the laboratory in the

appropriate manner, i.e. in a cooler at 4°C, and analysed within 24 hours of arrival. Sampling times and sites were in accordance with the intended purpose (Table I, Fig. 1).

Microbiological and physical/chemical analyses

Microbiological analyses

Culturable microorganisms

The enumeration of culturable microorganisms was carried out by inoculating Petri dishes containing yeast extract agar with a measured volume (1 ml) of the water sample to be analysed or dilutions of the sample, then incubating the dishes at $36 \pm 2^\circ\text{C}$ for 44 ± 4 h, in accordance with Moroccan standard ISO 6222 of 2007.

Total coliforms

The enumeration of total coliforms was carried out by filtering a volume of water to be analysed (100 ml) through a cellulose membrane filter (0.45 μm pore size), after the appropriate dilutions. The membrane was placed on lactose TTC (triphenyltetrazolium chloride) agar with Tergitol 7. Incubation was carried out at a temperature of $36 \pm 2^\circ\text{C}$ for 21 ± 3 h, in accordance with Moroccan standard ISO 6461-2 of 2007.

Faecal coliforms and *Escherichia coli*

In accordance with Moroccan standard ISO 6461-2 of 2007, the enumeration of faecal coliforms was carried out in the same way as for total coliforms, except that incubation was conducted at a temperature of $44 \pm 0.5^\circ\text{C}$ for 21 ± 3 h, then continued for 44 ± 4 h. Based on confirmed colonies of faecal coliforms, a representative number of the typical colonies obtained (at least 10) were sub-cultured on a non-selective agar (tryptophan broth), after which the tubes containing the tryptophan broth were incubated at $44 \pm 0.5^\circ\text{C}$ for 21 ± 3 h. Indole production was then controlled by adding 0.2–0.3 ml of Kovacs reagent. The appearance of red colouring on the surface of the broth confirmed indole production. Finally, all colonies with a

negative oxidase reaction but a positive indole reaction were considered to be *Escherichia coli*.

Faecal streptococci and intestinal enterococci

The enumeration of faecal streptococci was carried out by filtering a volume of water (100 ml), after the appropriate dilutions, through a membrane filter with a 0.45 µm pore size. The filter was placed on a solid Slanetz and Bartley selective medium and incubation was carried out at 37°C for 44 ± 4 h, in accordance with Moroccan standard ISO 7899-2 of 2007.

Confirmed colonies of faecal streptococci were then used to investigate the presence of intestinal enterococci. This stage consisted of transferring the membrane containing typical colonies of faecal streptococci onto an agar medium (bile esculin azide [BEA] agar) before incubating the Petri dishes at 44°C for a maximum of 2 hours.

Spores of sulphite-reducing anaerobes

To detect spores of sulphite-reducing anaerobes in a water sample of predetermined volume (100 ml), the first stage of the process was to select the spores by heating at 80°C for long enough (10 min) to destroy vegetative cells. The next stages were filtration and incubation. The water sample was filtered through a membrane filter (0.2 µm pore size). After placing the membrane on a tryptone sulphite cycloserine (TSC) agar medium, the Petri dishes were incubated at 37 ± 1°C for 20 ± 4 h and 44 ± 4 h. The count was considered to be the highest number of black colonies of the two readings (at 20 ± 4 h and 44 ± 4 h), in accordance with Moroccan standard ISO 6461-2 of 2007.

Physical/chemical analyses

The study also focused on the *in situ* measurement of: pH, using a portable pH meter (HANNA Hi 8519N); conductivity, using a portable conductivity meter (Consort K912); and water temperature, using a thermometer (electronic digital thermometer with a range from -50°C to 150°C). The other physical/chemical analyses were

performed in the laboratory using methods approved by Rodier *et al.* (9).

Table II summarises the methodology used to analyse the different parameters of water quality.

In parallel with this study, a questionnaire previously validated by poultry veterinarians covering a range of factors affecting water quality (including location and environment, water management, infrastructure, facilities, operation, hygiene, staff and feed) was completed in conjunction with farmers. The aim of the questionnaire was to determine potential risk factors for bacterial contamination of drinking water.

Statistical analysis

The statistical study focused on contamination with faecal coliforms on Day 60 (i.e. 60 days after placement of the poults), because there was a significant difference ($p < 0.001$) between the number of these bacteria at the main inlet to the water line of each turkey house on Day 0 (at poult placement) and the number at the end of the water line on Day 60 (Table III).

Furthermore, the presence of faecal coliforms may indicate the presence of enteric microorganisms, such as salmonella (13) or Norwalk virus (14). The values obtained were compared with: industry standards on the bacteriological quality of drinking water (10); American standards on drinking water quality for poultry (11); and European directives on drinking water (12).

A sample was deemed contaminated if the number of faecal coliforms exceeded the standard (5 per 100 ml). The variable is therefore dichotomous and describes contamination or non-contamination with faecal coliforms. To highlight the correlation between this variable and each explanatory variable, a chi-square (χ^2) test was performed and odds ratios were calculated with a 95% CI using the Statistica 6.0 software (Statsoft Ltd, Chicago, III). Questionnaire results were processed using the Sphinx Plus² software (version 4.5.0.19).

Results

Microbiological analyses

On Day 0 (poult start), all the samples collected at the main inlet to the water line were heavily contaminated with total bacteria (averaging $3.4 \pm 0.80 \log_{10}$ colony-forming units [cfu]/ml) and with coliforms (averaging $3.5 \pm 0.26 \log_{10}$ cfu/100 ml). All the samples analysed were therefore of unacceptable quality. The samples revealed heavy bacterial contamination with faecal coliforms, *E. coli*, streptococci and enterococci, which resulted in a rate of compliance with standards of 10–25% (Table III). The statistical analysis showed that there was no significant difference ($p < 0.05$) between the values recorded at the main inlet and at the end of the line on Day 0 (i.e. the day on which poults were placed in the turkey house) (Table III).

At the end of the line, 60 days after poult start (Day 60), the content of total bacteria, total coliforms, faecal coliforms and *E. coli* was significantly reduced ($p < 0.05$), whereas there was no significant difference in contamination with enterococci and streptococci between the main inlet on Day 0 and the end of the line on Day 60 (Table III).

Physical/chemical parameters

The physical/chemical analyses related to nine parameters. Table IV provides a summary of the results. A comparison of the physical/chemical parameter values with the standards shows that more than 90% of the drinking water samples were of satisfactory quality in terms of pH (6.4 ± 0.2), nitrite content (0.049 ± 0.13 mg/l), conductivity ($1,381.2 \pm 731.1$ μ s/cm), nitrate content (11.9 ± 4.3 mg/l) and iron content (0.03 ± 0.05 mg/l), whereas total hardness exceeded 50°f (the recommended standard) in six farms (30%). In 80% of the samples, the ammonium content of the water analysed did not exceed 0.5 g/ml.

Potential risk factors for water contamination with faecal coliforms at Day 60

Over the 60 days of the study, 11 of the 20 farmers carried out antibacterial water treatment by continuous chlorination using proportioning pumps (the desired concentration being 1–2 parts per million [ppm] residual chlorine at the drinker), whereas the remaining nine farmers carried out occasional chlorination. With a risk of 10%, a significant correlation was observed between chlorination frequency and contamination of drinking water in turkey houses with faecal coliforms ($p < 0.065$) (Table V). Indeed, only 2 of the 11 farms that had practised continuous chlorination during the production of each batch of birds were contaminated with faecal coliforms, whereas contamination exceeded the standard in six of the nine farms where the water had been chlorinated occasionally without using a proportioning pump (Table V).

Cleaning of equipment (including feeders, bins, drinkers, water lines and feed and water distribution systems) both during each production cycle and at cleanout (odds ratio = 14; 95% CI of 1.25–156.6) appears to have contributed significantly to reducing the risk of contamination. Indeed, 87.5% of farms where the equipment was cleaned both at cleanout and during production were not contaminated with faecal coliforms (Table V). The continuous administration of broad-spectrum antibiotics in water, starting on Day 1 (odds ratio = 6.82; $p = 0.001$), to control salmonella and colibacillosis, also proved to be an effective prophylactic for controlling coliforms in drinking water. However, pH (odds ratio = 4.57; $p < 0.32$, 95% CI of 0.41–51) and total hardness (odds ratio = 3.6, $p < 0.33$, 95% CI of 0.5–27) had no significant impact on the number of faecal coliforms found at the end of the line on Day 60 (Table V).

Discussion

Table III shows that, on Day 0, there is no significant difference ($p < 0.05$) between the number of bacteria at the main inlet (immediately after cleanout) and the number at the end of the water line. This is probably a result of effective cleaning and disinfection of

the houses at cleanout: after washing the house and equipment thoroughly, most farmers disinfect all interior surfaces of the house and all rearing equipment with disinfectants containing phenols, iodoform or quaternary ammonium compounds on surfaces free from organic matter. This indicates that heavy water contamination at the end of the line on Day 0 is caused by water contamination at source at the main inlet to the water line (or well). A significant reduction in flora at the end of the line ($p < 0.05$) on Day 60 (Table III) indicates that on-farm practices and antibacterial treatments (water chlorination, use of hydrogen peroxide to acidify drinking water) are reasonably effective but still inadequate. Indeed, as 100% of the farms surveyed use well water, heavy contamination of the water at source with total bacteria and total coliforms on Day 0 ($3.4 \pm 0.80 \log_{10}$ cfu/ml and $3.5 \pm 0.26 \log_{10}$ cfu/100 ml, respectively) could indicate poor protection of wells, 30% of which are open wells and 70% of which are not regularly maintained (drained every five years).

On Day 0, only 10% of the water samples complied in terms of *E. coli* and faecal coliforms. As these bacteria come exclusively from the intestines of warm-blooded animals, including humans, their presence in treated water points clearly to faecal contamination (15). The study confirmed this assumption, as there was a significant correlation ($r = 0.64$, $p < 0.01$) between total coliforms and *E. coli* on Day 60 at the end of the line at the last drinker. Indeed, other animals (such as cattle, birds, sheep and rats) had free access to 85% of the farms surveyed. This faecal contamination can also signal the presence of enteric microorganisms (15), reducing livestock performance (feed conversion ratio, average daily weight gain) within the house. Heavy contamination with *E. coli* at the end of the water line on Day 60 ($1.2 \pm 1.01 \log_{10}$ cfu/100 ml) poses a real threat to animal health, as confirmed by a more than 10% mortality rate found in 65% of the farms surveyed. Stordeur and Mainil (16) report that *E. coli* causes a number of infectious diseases. In addition, 80% of the samples exceeded the recommended standard in terms of streptococci ($> 5/100$ ml) on Day 60, resulting in an increased risk of gastroenteritis, even with a relatively small number of faecal streptococci (3–10 bacteria/100 ml) (17). This heavy contamination

also poses a health risk to farm workers, as they drink the same water as poultry.

This study has shown that pH has no significant effect on water contamination with faecal coliforms ($p = 0.32$) at the end of the line on Day 60. The pH of all 20 samples tended to be acid (5.8–6.6), which probably stems from the granitic soils through which the water passes, as the pH of natural water depends on the underlying geological formation of the land (18). It can also be influenced by oxygenation of the source, which affects pH. Poor oxygenation of the source due to the depth and seal of wells leads to water that is low in hydroxide ions (OH) and hence low in pH. As the intestinal environment of poultry is acid, the pH of drinking water should preferably be less than 7, because this has a stabilising and selective effect on commensal gut flora. However, water that is too acidic (< 5.5) corrodes water lines and is potentially harmful to the digestive and urinary tract (causing diarrhoeal syndromes and renal and urinary disorders) (13). Lastly, pH is a factor influencing drug molecules (acids or alkalis), which dissolve more or less completely depending on the chemical balance (19).

The results of this study have also shown hardness to have no significant effect on reducing the number of coliforms at the end of the water line ($p < 0.33$). Average water hardness across all the samples was 55.2°f, which is rather high. However, hard water interferes with the intestinal absorption of trace elements (18) and macro-elements and causes scale to form on watering equipment (20). It also promotes intestinal irritation, pecking and cannibalism.

The average concentration of chlorides was around 399.6 mg/l, with 55% of values exceeding the standard (250 mg/l). The abnormal levels observed in three farms (617.17, 696.15 and 1,462.5 mg/l) could indicate excessive use of chlorine, as these farms practise continuous chlorination with no proportioning or electric pump to allow for treatment at low concentrations (0.01–0.1% chlorine).

As regards salt intake in poultry feed, studies have shown that a normal level of salt in feed (0.3%) and a salt concentration of 4 ppm

in drinking water can cause chickens, turkeys and ducks to lower their water consumption, leading to a reduced feed intake, lower growth rate and higher mortality rate (21). In 52% of the study samples, water salinity exceeded 3,000 mg/l, with a maximum of 4,890 mg/l (Table IV), which, according to Casteel *et al.* (22), can cause loose droppings, increase mortality and reduce growth rates, especially in turkeys, and may even undermine the efficacy of drug treatments. A total of 20% of the farms exceeded the standard (0.5 mg/l) in terms of ammonium content, with maximum recorded levels of 5.17 mg/l. If ammonium is present and the water also contains a high concentration of nitrates (0.58 mg/l), this indicates bacterial evolution of forms of nitrogen, which are often associated with faecal contamination (2).

Conclusion

In the light of this study, farmers are advised to protect wells from contamination and to install water purification units (pre-oxidation, coagulation, flocculation, disinfection) in order to improve water quality in poultry farms. Farmers should also clean and disinfect turkey houses and feed silos scrupulously after each batch of birds in order to prevent the transfer of pathogens from one batch to the next. Another sensible measure would be to protect groundwater (by fencing off the area, preventing animals from grazing nearby to avoid faecal contamination, concreting the area, etc.). Lastly, to correct non-compliant physical/chemical parameters, farmers may acidify the water (to correct pH), use softeners (to correct hardness) and denitrify (to correct the nitrate and nitrite content), or reduce the salt content of feed (where water is highly saline).

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Table I
Microbiological and physical/chemical analyses according to intended purpose

Sampling	Number of samples	Type of analysis	Purpose
At the main inlet to the water line immediately after cleanout on the day the poultts arrive (Day 0)	20	Bacteriological	To determine the initial microbiological and physical/chemical quality of water
	20	Physical/chemical	
At the end of the line on Day 0 (last drinker)	20	Bacteriological	To assess the effectiveness of cleaning and disinfection of water lines at cleanout
At the end of the line on Day 60	20	Bacteriological	To measure the microbiological evolution of water

Table II
Methods used to analyse the different physical/chemical parameters of water quality (9)

Parameter	Measurement method	Unit
Chlorides	Mohr method	mg/l
Total hardness	EDTA (Ethylenediaminetetraacetic acid) titration	°f*
Iron and ammonium	Molecular absorption spectrometry	mg/l
Nitrates	Molecular absorption spectrometry	mg/l
Nitrites	Molecular absorption spectrometry	mg/l

* French degree: 10 mg of calcium carbonate per litre

Table III
Compliance rates, standards and averages in microbiological analyses (\log_{10} cfu/100 ml \pm standard deviation) of drinking water samples ($n = 60$) taken from the main inlet and at the end of the line (EL) in 20 broiler turkey farms

Values in the same line that do not exhibit a significant difference ($p < 0.05$) have been assigned the same letter (a or b)

Microorganisms sought	D0 main inlet	D0 EL	D60 EL	Standards*	Compliance rate	
					D0 main inlet	D60 EL
Total bacteria**	3.4 \pm 0.80 ^a	3 \pm 0.39 ^a	2.8 \pm 0.77 ^b	< 100 (per 1 ml)	0%	10%
Total coliforms	3.5 \pm 0.26 ^a	3 \pm 1.07 ^a	2.6 \pm 0.51 ^b	< 5 (per 100 ml)	0%	0%
Faecal coliforms	2.8 \pm 1.04 ^a	2.7 \pm 1.14 ^a	1.2 \pm 1.04 ^b	< 5 (per 100 ml)	10%	45%
<i>Escherichia coli</i>	2.5 \pm 0.94 ^a	1 \pm 1.49 ^b	1.2 \pm 1.01 ^b	0 (per 100 ml)	10%	35%
Faecal streptococci	1.7 \pm 0.56 ^a	1.9 \pm 0.84 ^a	1.7 \pm 0.96 ^a	< 5 (per 100 ml)	25%	20%
SRA	0.5 \pm 1.14 ^a	0.6 \pm 1.46 ^a	1.6 \pm 1.15 ^b	< 10 (per 100 ml)	80%	30%
Enterococci	1.3 \pm 0.43 ^a	1.1 \pm 0.74 ^a	1.3 \pm 0.43 ^a	0 (per 100 ml)	20%	15%

* Industry standards (10); American standards (11); European directives (12)

** Total bacteria are expressed as \log_{10} cfu/ml

SRA: sulphite-reducing anaerobes

D0 and D60: sampling dates for the analyses (0 and 60 days after poult placement)

Table IV
Averages and standards in the physical/chemical analysis of
drinking water samples ($n = 20$) taken from the main inlet to the
water line in 20 broiler turkey farms

Physical/chemical parameter	Descriptive analysis				Standard	Compliance rate
	Average	SD	Minimum	Maximum		
pH	6.4	0.2	5.8	6.6	6–7	90%
C	1,381.2	731.7	560	3,540	2,500 $\mu\text{s/cm}$	90%
Sal.	727.3	391.1	297	4,890	1,000–2,999 mg/l	25%
TH	55.2	27.8	26.2	138.5	< 50°f	65%
NO ₂ ⁻	0.049	0.13	0	0.58	< 0.1 mg/l	90%
CL	399.6	300.7	99.4	1,462.5	< 250 mg/l	45%
NH ₄ ⁺	0.45	1.12	0	5.17	< 0.5 mg/l	80%
NO ₃ ⁻	11.92	4.3	2.5	16	< 50 mg/l	100%
Iron	0.03	0.05	0	0.22	< 0.3 mg/l	100%
T	23.1	1.6	19	25	—	—

C: conductivity ($\mu\text{s/cm}$)

Cl: chloride (mg/l)

Fe: iron (mg/l)

NH₄⁺: ammonium (mg/l)

NO₂⁻: nitrite (mg/l)

NO₃⁻: nitrate (mg/l)

Sal.: salinity (mg/l)

SD: standard deviation

T: temperature (°C)

TH: total hardness (CaCO₃ or mg/ml)

Table V
Potential risk factors for water contamination with faecal coliforms (chi-square [χ^2] test with a significance level of 5%) in broiler turkey farms on Day 60

Variable	Percentage of contamination	P α	χ^2	OR	95% CI (OR) ^(a)	RR
Chlorination						
Continuous	18.2	< 0.065	4.848	9	1.14–71	3.67
Occasional	66.6					
Cleaning of equipment						
At cleanout	66.6	< 0.028	5.69	14	1.25–156.6	5.33
During the flock life cycle and at cleanout	12.5					
pH						
6.5–8	80	< 0.32*	1.68	4.57	0.41–51.1	1.71
< 6.5	46					
Total hardness						
≤ 50°f**	64.3	< 0.33*	1.62	3.6	0.5–27	1.9
> 50°f	33.3					
Antibiotic treatment at poult placement						
Yes	30.5	< 0.001	11.4	6	2.1–35.2	3.56
No	75					

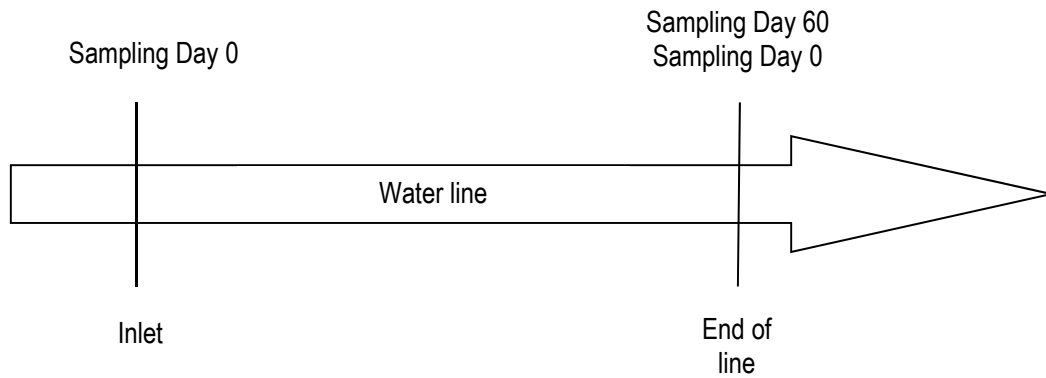
OR: odds ratio

RR: relative risk; $p < 0.05$ and $p < 0.1$: variables significantly associated with water contamination with faecal coliforms at 95% and 90%

a) 95% confidence interval of an odds ratio according to Woolf's method (90% CI for chlorination)

* Statistically non-significant link

** French degree: 10 mg of calcium carbonate per litre



D0 and D60: sampling dates for the analyses (0 and 60 days after poult placement)

Fig. 1
Study implementation by sampling points