# **Chapter 4 – Results dataset**

All data has been electronically stored in the Department of Plant and Soil Sciences.

# ***Cryptosporidium* Prevalence in Water, Soil, and Fresh Produce**

### **4.3.1 Occurrence of *Cryptosporidium* in water samples**

*Cryptosporidium* was not detected using real-time PCR, however, out of 22 processed water samples, three samples (13.6%) tested positive for *Cryptosporidium* oocysts using ddPCR. The farms utilised different water sources: three farms used borehole water, three used surface water, and one relied on municipal supply. Contamination was detected only at the water sources of Farm C (borehole water) in 50% (1/2) of the samples and Farm E (surface water from a dam) in 100% (2/2) of the samples; *Cryptosporidium* was not detected at the irrigation points (Figure 4.3).

**Figure 4.3:** Proportion of *Cryptosporidium* contaminated water samples.

### **4.3.2 Occurrence of *Cryptosporidium* in soil**

*Cryptosporidium* was not detected using real-time PCR, however, out of 90 processed soil samples, 21 (23.3%) tested positive for *Cryptosporidium* oocysts using ddPCR, which included six of the seven farms tested. The percentage of positive samples based on different fertiliser amendments—manure, plant-based, commercially available (chemical), or untreated—is illustrated in Figure 4.4. The highest number of positive samples was observed in soil amended with a combination of commercial fertiliser and manure (n=9, 10%), followed by manure only (n=6, 6.3%), plant-based (n=4, 4.4%), and untreated soil (n=2, 2.1%). Overall, Farm G had the highest number of samples that tested positive for *Cryptosporidium* (3/5, 60%), followed by Farm F (6/15, 40%), with both farms using a combination of manure (chicken and cow, and chicken, respectively) and chemical fertiliser. Farm E showed a lower number of samples testing positive (5/15, 33.3%), followed by Farm C (4/15, 26.7%), Farm D (2/20, 10%), and Farm B (1/15, 6.7%). No soil samples tested positive on Farm A. The concentration of *Cryptosporidium* oocysts in soil from each farm is shown in Table 4.3.

**Figure 4.4:** Proportion of soil samples contaminated with *Cryptosporidium* on each farm.

**Table 4.3:** Occurrence and concentration of Cryptosporidium oocysts in soil from each farm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sampling area** | **Province** | **Type of Fresh Produce** | **Total number of samples** | **Percentage of total “Positive”**  **Samples** | **Average *C. parvum***  **copies/26ɥl reaction (ddPCR)** |
| **Farm A** | Gauteng | Soil (Kale field) | 5 | 0% | - |
| **Farm B** | Gauteng | Soil (Chinese spinach field) | 5 | 20% | 0.025 |
| Soil (Rape field) | 5 | 0% | - |
| Soil (Kale field) | 5 | 0% | - |
| **Farm C** | Gauteng | Soil (Lettuce field) | 5 | 40% | 0.029 |
| Soil (Radishes field) | 5 | 20% | 0.016 |
| Soil (Rocket field) | 5 | 20% | 0.016 |
| **Farm D** | Northwest | Soil (Baby carrots field) | 5 | 20% | 0.014 |
| Soil (Rocket field) | 5 | 0% | - |
| Soil (Spring onions field) | 5 | 0% | - |
| Soil (Leeks field) | 5 | 20% | 0.015 |
| **Farm E** | Limpopo | Soil (Green peppers field) | 5 | 20% | 0.032 |
| Soil (Tomatoes field) | 5 | 60% | 0.204 |
| Soil (Spring onion field) | 5 | 20% | 0.031 |
| **Farm F** | Gauteng | Soil (Green peppers field) | 5 | 40% | 0.031 |
| Soil (Spinach field) | 5 | 20% | 0.031 |
| Soil (Onions field) | 5 | 60% | 0.049 |
| **Farm G** | Gauteng | Soil (Peas field) | 5 | 60% | 0.178 |

### **4.3.3 Occurrence of *Cryptosporidium* on fresh produce**

*Cryptosporidium* was not detected using real-time PCR, however, out of 98 processed fresh produce samples, 34 (34.7%) tested positive for *Cryptosporidium* oocysts using ddPCR in samples from all seven farms. A total of 14 fresh produce types were examined, with 13 types testing positive (Table 4.4). The highest number of positive samples was observed in radish (5/5, 100%), followed by tomato (4/5, 80%), peas (3/5, 60%), rocket (5/10, 50%), baby carrots (2/5, 40%), onions (2/5, 40%), spring onion (4/10, 40%), kale (3/10, 30%), spinach (4/15, 26.67%), lettuce (2/8, 25%), leeks (1/5, 20%), and green pepper (1/10, 10%). No contamination was detected in rape samples. The concentration of *Cryptosporidium* oocysts in different fresh produce types from each farm is illustrated in Figure 4.5, categorised as follows: leafy green vegetables (kale, spinach, rape, lettuce, rocket, spring onion, leeks), fruiting vegetables (tomato, green pepper, peas), and root vegetables (radish, baby carrots, onion).

**Table 4.4:** Occurrence and concentration of Cryptosporidium oocysts of each type of fresh produce from each farm

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sampling area** | **Province** | **Type of Fresh Produce** | **Total number of samples** | **Percentage of total “Positive”**  **Samples** | **Average *C. parvum***  **copies/26ɥl reaction (ddPCR)** |
| **Farm A** | Gauteng | Kale | 5 | 60% | 0.059 |
| Spinach | 5 | 20% | 0.015 |
| Lettuce | 3 | 0% | - |
| **Farm B** | Gauteng | Chinese spinach | 5 | 20% | 0.045 |
| Rape | 5 | 0% | - |
| Kale | 5 | 0% | - |
| **Farm C** | Gauteng | Lettuce | 5 | 40% | 0.033 |
| Radishes | 5 | 60% | 0.051 |
| Rocket | 5 | 0% | - |
| **Farm D** | Northwest | Baby carrots | 5 | 40% | 0.038 |
| Rocket | 5 | 100% | 0.081 |
| Spring onions | 5 | 40% | 0.029 |
| Leeks | 5 | 20% | 0.018 |
| **Farm E** | Limpopo | Green peppers | 5 | 0% | - |
| Tomatoes | 5 | 80% | 0.131 |
| Spring onion | 5 | 40% | 0.053 |
| **Farm F** | Gauteng | Green peppers | 5 | 20% | 0.015 |
| Spinach | 5 | 40% | 0.034 |
| Onions | 5 | 40% | 0.049 |
| **Farm G** | Gauteng | Peas | 5 | 60% | 0.154 |

**Figure 4.5:** Proportion of *Cryptosporidium* contamination by fresh produce type, including leafy green vegetables (kale, spinach, rape, lettuce, rocket, spring onion, leeks), fruiting vegetables (tomato, green pepper, peas), and root vegetables (radish, baby carrots, onion) across each farm.

### **4.3.4 Comparison of the occurrence of positive samples in water, soil, and fresh produce across different regions**

The frequencies of *Cryptosporidium* based on the farming production practices are displayed in Table 4.5. For this scoping study, certain trends were observed between type of farming practices, water source type, soil amendment, and fresh produce type. However, the number of samples was not enough for a comprehensive statistical analysis. Given the limited sample sizes, the ranking of contamination rates provides preliminary insights rather than statistically definitive conclusions, and further studies with larger sample sizes are required to confirm potential trends with greater statistical confidence.

**Type of Farming:** The analysis indicates that organic farming had the highest number of positive samples (10/32, 31.3%), followed by conventional farming (23/80, 28.75%) and integrated farming systems (25/98, 25.5%).

**Water Source Type:** The groundwater samples showed a lower percentage of positive samples (1/6, 16.7%) compared to surface water used for irrigation purposes (2/7, 28.6%). Notably, none of the municipal water samples tested positive for *Cryptosporidium*.

**Soil Amendment:** Soil treated with a combination of commercial fertiliser and manure exhibited the highest number of positive samples (9/20, 45%), while soil amended solely with manure had 17.1% (6/35) of samples testing positive. This could indicate that the use of manure may introduce or increase the presence of pathogens like *Cryptosporidium*. Additionally, soil treated with plant-based organic fertilisers (from composting) had 26.7% (4/15) of samples testing positive, while untreated soil had the lowest number of positive samples (2/15, 10%).

**Type of Fresh Produce:** The percentage of samples positive for *Cryptosporidium* was different among the different types of fresh produce (Table 4.5). Root vegetables had the most samples positive (7/15, 46.7%), followed by fruiting vegetables (8/20, 40%), while the lowest number of positive samples were from the leafy green vegetables group (19/63, 30.15%).

**Table 4.5:** Detection of Cryptosporidium in water, soil, and fresh produce samples from small-scale farms according to the different production practices used

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Variable** | **“Positive” samples (n)/Total samples** | **Percentage of total “Positive”**  **Samples** |
| **Type of farming** | Integrated (Aquaponics and animal husbandry) | 25/98 | 25.5% |
| Conventional | 23/80 | 28.75% |
| Organic | 10/32 | 31.3% |
| **Total samples (farming type)** |  | 58/210 | 27.62% |
| **Water source type** | Groundwater | 1/6 | 16.7% |
| Surface water | 2/7 | 28.6% |
| Municipal water | 0/2 | 0% |
| **Total samples (water source)** |  | 3/15 | 20% |
| **Soil amendment** | Combination of commercial fertiliser and manure | 9/20 | 45% |
| Manure | 6/35 | 17.1% |
| Organic fertiliser | 4/15 | 26.7% |
| No amendment | 2/20 | 10% |
| **Total samples (soil amendment)** |  | 21/90 | 23.3% |
| **Type of vegetable** | Leafy green vegetable | 19/63 | 30.15% |
| Root vegetable | 7/15 | 46.7% |
| Fruiting vegetable | 8/20 | 40% |
| **Total samples (vegetable type)** |  | 34/98 | 34.7% |